ARMY PUBLIC HEALTH CENTER (Provisional)

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Toxicology Study No. S.0024883

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

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Toxicology Portfolio
Health Effects Research Program
Army Public Health Center (Provisional)

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14. ABSTRACT

The U.S. Army is engaged in an effort to develop industrial processes that have less impact upon human health and the environment in order to improve sustainability in the Army industrial base and to protect the health of Soldiers and civilian workers. Methylnitroguanidine is under evaluation as a replacement for legacy munitions such as TNT and RDX and is a component of the munitions mixture DEMN. The objectives of this study were to determine the oral acute and subacute toxicity of MeNQ in the rat. MeNQ was not acutely toxic, with no mortalities observed in either male or female rats up to the limit dose of 2000 milligrams/kilogram (mg/kg) body weight in the acute test. There were also no signs clinical signs of toxicity or morbidity in the subacute 14-day study up to 1250 mg/kg-day, the highest dose tested in both males and females. As no signs of toxicity were observed, it was not possible to derive a benchmark dose. These tests indicate that MeNQ has low toxicity over a short exposure time frame and can be considered as a replacement for legacy munitions..

15. SUBJECT TERMS

Methylnitroguanidine, MeNQ, rat, oral, acute, micronucleus, toxicology

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Protocol No. 30-14-07-01
Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Data Requirement

Health Effects Testing Guidelines Reference No. OPPTS 870.3050: Repeated Dose 28–Day Oral Toxicity Study in Rodents

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Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The statistical analyses of the data were conducted by the U.S. Army Public Health Command statisticians. It is not known if these analyses were conducted in accordance with Good Laboratory Practice Standards.

2. During the acute study, only one dosing suspension was analyzed for concentration. This was the repeat of the maximum tolerated dose and was confirmed to be within acceptable limits. Other suspensions were prepared in the same manner.

3. The test article characterization (purity) was conducted by the manufacturer and it is not known whether the testing was done in compliance with the above regulation.

4. Homogeneity of the test substances was not conducted. Samples were always well mixed at the time dosing began and were pulled from the center of the dosing suspension.

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Biologist
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19 Apr. 2016
Date

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EFFECTS OF ACUTE AND SUBACUTE ORAL METHYLNITROGUANIDINE EXPOSURE TO RATS (*Rattus norvegicus*).
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1 Summary

1.1 Purpose

These studies were conducted in order to determine the oral toxicity of methylnitroguanidine (MeNQ). The goals were to determine the median lethal dose (LD_{50}) and 95 percent confidence intervals from an acute oral administration of MeNQ and to determine the health effects, including analysis of sperm and assessment of compound genotoxicity using the micronucleus assay (males only) of repetitive oral exposure to MeNQ in male and female rats.

1.2 Conclusions

Daily oral exposures of MeNQ to male and female rats at dosages of 1250 mg/kg-d in corn oil for 14 days did not induce any signs of toxicity. There were no changes in body mass, body mass gain, clinical chemistry, hematology, organ weights or weight ratios, incidence of micronucleated reticulocytes, or effects on sperm motility or number at any of the dosages. There were no overt clinical signs in the acute or subacute dosing periods. Microscopic analysis of the tissues revealed no significant effect on any of the tissues examined at the highest dosage.

Under the study conditions, oral administration of a single dose of MeNQ up to 2000 mg/kg or dosages up to 1250 mg/kg-d MeNQ for 14 days did not produce any toxic effects. As the study did not result in determining a lethal exposure level, no LD_{50} could be calculated.

2 References

See Appendix A for a listing of references.

3 Authority

Military Interdepartmental Purchase Request (MIPR) No. 0010493597. This study was conducted with funding from the Environmental Acquisition and Logistics Sustainment Program of the U.S. Army Research Development and Engineering Command (USARDECOM). This toxicology study addresses, in part, the environmental safety and occupational health requirements outlined in Army Regulations (AR) 200-1, AR 40-5, and AR 70-1; Department of Defense Instruction 4715.4; and Army Environmental Requirements and Technology Assessments (Department of the Army (DA), 2007a and b; DA, 2003; Department of Defense (DOD), 1996; and U.S. Army Environmental Command (USAEC), 2009). It was performed as part of an on-going effort by the U.S. Army Environmental Quality Technology (EQT), Ordnance Environmental Program Pollution Prevention

Team, to produce safer ordnance. This program is under the direction of the USARDECOM Environmental Acquisition Logistics & Sustainment Program and EQT Pollution Prevention.

4 Background

Historically, the development of novel munitions was based on the efficacy of the compound, with few and inconsistent data collected to evaluate occupational and environmental impacts of the munition. Toxicity screening of a novel munition was not required to be completed prior to implementation. As a result, the health and environmental impacts of the legacy munitions remained undefined until recently, leading to a high cost of remediation efforts [1]. Moving forward, the adoption of new munitions must be grounded both in efficacy and scientific support for reduced negative environmental and occupational health effects. Thus, it is the task of the Army Public Health Center (APHC (Prov)) to determine the toxicity of candidate replacement munitions, in a manner consistent with the research, development, testing and evaluation levels of munition development [1]. For an effective force, the munitions that are used to train U.S. Army Soldiers must be the same as those that are utilized in the field. By evaluating candidate munitions early in the development process, budget expenditures related to unnecessary training and remediation can be alleviated.

MeNQ is a white powder and is currently under evaluation by the U.S. Army Research, Development, and Engineering Command (RDECOM) as a potential component in propellant/warhead formulations to replace 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) based formulations. The compound was not found to be acutely toxic at 1000 mg/kg in male and female Fischer 344 rats, nor did it cause skin irritation in female New Zealand white rabbits, however, it did cause mild conjunctival irritation [2]. Testing of MeNQ in a guinea pig indicated that MeNQ is a weak skin sensitizer. There is no *in vitro* evidence that the compound is mutagenic. MeNQ can also be found as a metabolic product of clothianidin, one of the recently developed neonicotinoid insecticides [3]. There is some evidence that clothianidin exposure in mice can impact spermatogenesis and testes health [4].

The following report details the methodologies, findings and conclusions of a series of two oral toxicity studies performed with MeNQ in laboratory rats. The first study consisted of a sequential stage-wise probit (SSWP) acute test, while the second was a 14-day repeated dose study. These types of studies can be used to identify effect levels, define target organs, support regulatory actions, and provide risk assessment information.

The following table identifies the critical dates of this study.

Table 1. Critical Study Events

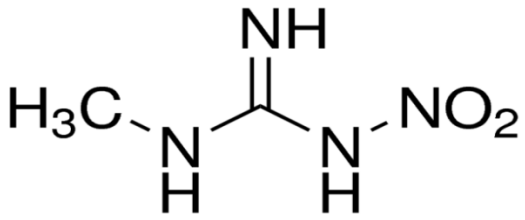
Critical Event	Date of Event
Protocol Approved	02 July 2014
Acute Animals Received	16 July 2014
Acute Experimental Start	22 July 2014
Acute Necropsies	5 August 2014; 7 August 2014
Acute Experimental Completion	7 August 2014
14-Day Animals Received	6 August 2014
14-Day Experimental Start	12 August 2014
Received Final Histopathology Report	3 March 2016
Study Completion	19 April 2016

5 Materials

5.1 Test Substance

MeNQ is a white energetic powder with a chemical abstract number of: 4245-76-5. It is flammable when wet and explosive when dry, it was therefore maintained as it was received from the manufacturer, which was wet (30.8% water; Sigma SDS). All dosing suspensions were prepared accounting for the water weight such that the target concentration of anhydrous MeNQ was achieved. The chemical structure is seen in figure 1. The material was acquired from Sigma-Aldrich, lot number MKBH7676V (St. Louis, MO) with a secondary standard acquired from Santa Cruz Biotechnologies (Santa Cruz, CA). For both the acute and subacute portions of the study, the compound was mixed with corn oil (Mazola®, commercially available). For each dosing suspension, the amount of material necessary was calculated and weighed on an analytical balance, transferred to a clean glass jar and suspended in the appropriate volume of corn oil. Each solution was stirred with a magnetic stir bar until a homogenous suspension was achieved. Dosing suspensions were allowed to settle between dosing periods, but were stirred for at least 30 minutes prior to dosing each day in order to achieve suspension homogeneity. Dosing suspensions were stirred throughout the dosing period. All dosing suspensions were prepared at least 3 days prior to use. A stability test was performed on MeNQ by Laboratory Sciences Portfolio (LAB) from a 10 mg/mL suspension, which indicated that MeNQ in corn oil was stable for at least one month at room temperature. Dosing suspensions were stored at room temperature when not in use.

Figure 1: MeNQ Structure



5.2 Animals*†

Adult Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA) were utilized for all studies. For the acute study, male and female rats were approximately 7 weeks of age at receipt into the animal facility, while male and female rats in the subacute study were approximately 8 weeks of age at receipt. Animals were examined and weighed by the Attending Veterinarian or their designee upon arrival into the facility and were found to be in acceptable health. All animals were allowed a 5-day period for acclimation into the facility. They were maintained in a temperature-, relative humidity-, and light-controlled room. Conditions were within the target ranges of 64-79 ^{0}F, 30 to 70 percent relative humidity with a 12-hour light/dark cycle [5], with the exception of a short (several hour) period where the humidity was outside the targeted range, but it was not considered to have compromised the integrity or validity of the study results. Animals were fed a certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and supplied with drinking quality water. Food and water were available *ad libitum* except during pre- and post-exposure fasting periods in the acute study and pre-necropsy in the subacute study. Rats were pair-housed except during the post-dosing observation period in the acute portion of the study. Animals were housed in suspended polycarbonate boxes on ventilated racks with Harlan Diamond Dry® bedding. Each rat was uniquely identified by number using cage cards and tail marking with indelible ink.

5.3 Contract Studies

The micronucleus analysis was performed by Litron Laboratories (Rochester, NY).

5.4 Additional In-House Analyses

The 14-day study included in-house histopathologic analysis of the study tissues and sperm analysis; the contributing scientist reports for these two analyses can be found in Appendices L and M respectively

5.5 Quality Assurance

The APHC (Prov) Quality Systems and Regulatory Compliance Office (QSARC) audited critical phases of this study. Appendix B provides the dates of these audits, the phases audited, along with the dates that the results of the inspections were reported to the Study Director and Management.

5.6 Study Personnel

Appendix C contains the names of persons contributing to the performance of this study.

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* Research was conducted in compliance with DOD and federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC 1996.

† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

6 Methods

6.1 General Description

The study consisted of an acute and repeated-dose oral toxicity tests and was conducted in a manner consistent with the methods outlined in the U.S. Environmental Protection Agency (USEPA) Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity Studies in Rodents [6]. Dose selection was initially based on the findings obtained in a previously conducted study at a different facility. In order to determine the LD_{50} with 95 percent confidence intervals of MeNQ, a sequential stage-wise probit (SSWP) was used with two separate stages of dosing [7, 8]. Dosing levels for the 14-day repeated dose test were determined following the completion of the acute oral toxicity test.

6.2 Sequential Stage-Wise Probit

Thirty male and female Sprague-Dawley rats, seven weeks old at receipt were obtained from Charles River labs. Prior to dosing, all animals were fasted overnight. Fasting was continued for four hours after dosing. Doses for the first stage of the MeNQ acute test were set at 61, 195, 625, and 2000 mg/kg. All doses were calculated based on bodyweights taken immediately prior to dosing. One male and one female rat were orally gavaged with the MeNQ/corn oil suspension at each dosage level using a 16 gauge x 2 inch stainless steel gavage needle. Two MeNQ/corn oil suspensions (20 and 200 mg/mL) were mixed to maintain the dosing volume below 10 mL/kg. In the second stage, an additional four male and four female rats were dosed with 2000 mg/kg using a single nominal 200 mg/mL suspension in corn oil.

Following administration of MeNQ in each stage, rats were held for a 14-day observation period. All clinical signs or incidences of death were recorded on a daily basis. Individual body weights were recorded on selected post-exposure days (days 3, 7 and 14 after dosing). All surviving animals were euthanized on day 14 via carbon dioxide asphyxiation and underwent gross necropsy. The estimated LD_{50} value and 95 percent confidence intervals were calculated using a probit analysis following the post-exposure observation period for the final stage of dosing.

6.3 14-Day Repeated Dose Toxicity Study

Upon completion and analysis of the data for the SSWP, a 14-day range-finding oral toxicity study was conducted in male and female rats. The range of dosing concentrations for the 14-day study was determined from the results of the acute study.

Sixty female and 60 male Sprague-Dawley rats, approximately 8 weeks of age at receipt, were used for this phase of the study. Animals were acclimatized to the facility for a period of 5 days prior to initiation of the study. Rats were randomized by body weight into 5 treatment groups and one corn oil control group (10 rats/dose group). Dosage levels were set at 100, 210, 415, 830 and 1250 mg/kg-day. The start dates for the study were staggered over a period of 6 days to facilitate the scheduling of necropsies and sample collection for the micronucleus assay and sperm analysis (described later). Animals were administered doses at 7 mL/kg. In order to facilitate this dosing

volume, 5 separate dosing suspensions were mixed (14.28, 30, 59.28, 118.57, 178.57 mg/mL). Animals were orally dosed daily for 14-days with a 16 gauge x 2-inch stainless steel gavage needle. The suspensions were sampled and analyzed to verify concentrations prior to dosing. Additional dosing suspensions were mixed and analyzed for concentration as needed.

Rat bodyweights were recorded on days 0, 1, 3, 7, 13 and 14. Rats were fasted overnight prior to necropsy on day 14. Daily observations and recordings for signs of toxicity and morbidity were taken for all animals. Observations consisted of each rat being removed from its home cage, handled and observed. Observations included, but were not limited to, evaluation of skin, fur, eyes and mucus membranes, respiratory and circulatory effects, autonomic effects, including salivation, central nervous system effects, including tremors and convulsions, changes in activity levels, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or abnormal behavior. Rats that died or were euthanized during the course of study were submitted for gross necropsy.

In the male group, 5 animals from the 3 highest dose groups without a mortality and the corn oil control group (n = 20) were randomly selected for tail-vein blood collection prior to necropsy for micronucleus analysis. Blood collection was conducted during the morning of necropsy, prior to anesthetization for intracardiac stick.

6.4 Micronucleus Assay

Testing and sampling procedures for the micronucleus assay followed the methodology outlined in the protocol provided in the MicroFlow Basic Kit® (Litron Laboratories, Rochester, NY 14623). Sixty to 120 µL of peripheral blood was drawn from the tail-vein of 5 selected rats in each of the three highest dose groups and from the corn-oil control group on day 14 of the sub-acute study. An additional 5 rats were bled 5 to 7 days prior to necropsy to serve as negative controls. These rats were then treated with 200 mg/kg EMS (CAS 62-50-0) in water 48, 24, and 4 hours prior to blood sampling and euthanasia. Blood samples were collected directly into syringes containing an anticoagulant provided by in the MicroFlow Basic Kit®.

Blood samples on each day of necropsy were fixed and processed within 5 hours of collection. Samples from the syringes were transferred to a 1.5 mL Eppendorf tube and briefly vortexed. 180 µL of sample was transferred to a labeled 15 mL conical tube containing 2 mL of ultracold (-75 to -85 ^{o}C) methanol. Two samples per rat were taken. The conical tube was recapped, briefly vortexed and immediately transferred to the -80 ^{o}C freezer. Samples were transferred into LTSS 5-6 days after fixation and sent to Litron Laboratories for flow cytometric analysis. See Appendix M for methodology of sample analysis and report.

6.5 Sperm Analysis

See Appendix N for the contributing scientist report.

6.6 Necropsy

At the conclusion of the in-life portion of the study (day 14), rats were anesthetized with CO_2, blood collected by intracardiac puncture, and euthanized using CO_2. Clinical chemistry and hematology

values were determined from all valid blood samples. Tissues for histological assessment were taken, including the brain, heart, liver, kidneys, spleen, adrenals, thymus, uterus, ovaries, testes and epididymides. All organs were weighed for absolute organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios. Organ weights were recorded on an electronic necropsy spreadsheet. Gross necropsy was completed on all animals.

The following parameters were analyzed as compared to the control group:

1. Body weights
2. Weight gains
3. Absolute organ weights
4. Organ-to-body weight ratios
5. Organ-to-brain weight ratios

6.7 Clinical Chemistry and Hematology

Blood was obtained from CO_2 anesthetized adult animals via intracardiac puncture at the termination of the study. Blood for clinical chemistry analyses was transferred to tubes with no anticoagulant, allowed to clot for at least 20 minutes, and centrifuged to obtain serum. Blood for hematology analyses was transferred immediately to tubes containing tripotassium ethylenediamine-tetraacetic acid (K_3EDTA). Animals were fasted overnight prior to blood collection.

Clinical Chemistry parameters including: albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (non-fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), inorganic phosphorous (PHOS), total bilirubin (TBIL), triglycerides (TRIG) total protein (TP), sodium (Na), potassium (K), and chloride (Cl) were determined (VetTest 8008 Chemistry Analyzer and Na, K, Cl VetLyte Analyzer, IDEXX Laboratories, Inc., Westbrook, ME) on all valid serum samples.

Hematology parameters including: white blood cell count (WBC), WBC differential (% neutrophils (NEU %N), % lymphocytes (LYM %L), % monocytes (MONO %M), % eosinophils (EOS %E), % basophils (BASO %B)), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV) and prothrombin time (PT sec) were determined (Cell-Dyn 3700 Hematology Analyzer, Abbott Laboratories, Abbott Park, IL) on all valid samples.

6.8 Histopathology

Tissues were appropriately preserved in 10 percent buffered formalin, selectively trimmed and placed in cassettes labeled with protocol number and animal identification number. Testes and

epididymides were preserved in modified Davidson's fixative for a period not exceeding 24 hours and were then transferred to 70 percent ethyl alcohol. Cassettes were placed in labeled formalin-filled bottles and processed in-house (See contributing scientist report, Appendix L). Tissues were routinely processed and paraffin embedded. All processed and embedded tissues were sectioned at 5 µm thick and automatically stained with hematoxylin and eosin. The pathologist examined slides for compound-induced histopathologic changes via light microscopy. The prevalence and severity of findings were graded as compared to controls. Findings were assigned as none, minimal, mild, moderate, or severe.

6.9 Statistical Analysis

Statistical assistance was provided in the analysis of body weights (Appendix O). Dose groups for all data collected at the termination of the study (organ weights, clinical chemistry, and hematology) were compared by a one-way analysis of variance (ANOVA). For organ weights, in addition to absolute weight, organ to brain and organ to body weight ratios were calculated and analyzed in a similar manner. Micronucleus assay data were also analyzed by a one-way analysis of variance. A *t*-test was performed to verify that the positive control group was significantly different from the negative control group. If there was a significant effect ($p \leq 0.05$) by dose groups, a Tukey's multiple comparison test was performed if variances were similar. If variances were unequal, a Dunnett's T3 test was utilized. Variance equality was determined by Levene's Test. Additionally, residuals for the test were saved and plotted on a Q-Q plot as an additional measure of variance. If both the Levene's test was significant and the Q-Q plot was not acceptable, data were log transformed and analyzed by ANOVA. SPSS 21.0 was used to perform all analyses.

7 Results

7.1 Analytical Results

MeNQ was analyzed for stability in solution over a 4 week period, performed by USAPHC LAB prior to and concurrently with the 14-day repeated dose study. The initial sampling period began three weeks prior to the initiation of study, which would provide the absolute minimal period of time that the MeNQ would need to be stable in corn oil. As multiple suspensions had to be made because of the consistency and malleability of the compound, no more than two weeks stability was necessary. The MeNQ concentration did not test outside of the acceptable range over the 4 week period. Recovery was no lower than 92 percent of the expected value during the sampling period. In the acute portion of the study, a single high dose suspension was tested, and was found to be within the acceptable range, at a value of 95 percent of expected. The MeNQ concentrations of the 5 dosing suspensions for the 14-day study were verified by APHC (Prov) LAB prior to use. A second sampling was completed when additional dosing suspensions were required. Dosing suspensions were considered to be acceptable if they fell within the acceptable limits of 70-130 percent of expected concentrations. All but one of the samples fell within 15 percent of the expected values (85% of expected or greater). The sample that did not fall within 15 percent of expected values fell to 78 percent of the expected concentration, this was still within acceptable limits. All samples were analyzed by liquid chromatography with an ultraviolet detector. All results are reported according to the nominal dosing concentration.

7.2 Sequential Stage-Wise Probit

All 16 rats (n = 8 male and 8 female) exposed to concentrations up to 2000 mg/kg MeNQ in corn oil survived throughout the 14-day recovery period. Therefore, the LD_{50} of MeNQ in both male and female rats is greater than 2000 mg/kg. As an LD_{50} was not achieved, confidence intervals are not reported. Animals gained weight throughout the 14-day observation period.

The only clinical observation during the 14-day observation period was a dried red material around the nose of one male in the limit dose (2000 mg/kg) dose group. This observation was not attributed to the administration of the test material, and could have been caused by chewing the red plastic enrichment tubes. Following the end of the 14-day observation period, all 16 rats underwent gross necropsy. One male rat in the 2000 mg/kg dose group had slightly mottled kidneys. Mottled kidneys are not necessarily an indicator of renal toxicity as a result of compound exposure and can be observed in healthy adult rats. As the gross necropsies did not reveal any gross lesions of concern, no target organs for the 14-day subacute study were identified in the acute study. See Appendix E for data pertaining to the acute study.

7.3 14-Day Oral Repeated Dose Toxicity Study

7.3.1 Mortality

There was one unscheduled pre-term death during the 14-day study. One male in the corn oil control group experienced a perforated esophagus during the oral gavage procedure and was euthanized immediately upon diagnosis of the event. The remainders received 14 daily doses and were euthanized for necropsy on the 15th day of the study.

7.3.2 Clinical Observations

All animals were observed at least once daily during the 14-day exposure period. The bulk of the clinical signs that were observed throughout the exposure period were related to alopecia or barbering. A total of 3 animals showed alopecia (2 males and 1 female) while another was barbering its front paws. Neither barbering nor alopecia are likely to be a result of compound exposure, but more likely in response to cage-mate altercations or the stress of dosing itself. One other male had a scar from a cage-mate fight on its back, while a female had a minor congenital hip abnormality that altered its gait, but did not affect the ability to access food or water or to move around the cage. See Appendix F for all observations throughout the 14-day study.

7.3.3 Body Mass and Mass Changes of Rats

Body weight gain across all the dose groups was similar throughout the 14-day exposure period. There were no statistically significant differences across dose groups at any point during the study. All animals gained weight similarly throughout the exposure period. See Appendix G for individual body weight data and analysis.

7.3.4 Clinical Chemistry and Hematology

No major effects on clinical chemistry parameters were found in either male or female rats in any dose groups. There were significant differences in some clinical chemistry parameters between dose groups in both the males and females; however none of these were between the dose group and controls. See Appendix H for the data indicating these differences.

7.3.5 Hematology

In the female dose groups, there was an increase in the mean percent of lymphocytes ($P = 0.002$), with the three highest dose groups significantly different from controls. No major effects on the hematology parameters in males were noted. See Appendix I for the hematology data and analysis.

7.3.6 Prothrombin Time

No significant dose group differences were observed for prothrombin time, see Appendix J for analysis.

7.3.7 Organ Mass and Ratios

There were no major effects of MeNQ administration on organ mass or ratios in any of the dose groups as compared to control groups. See Appendix K for organ mass data and analysis.

7.3.8 Micronucleus Assay

There was no significant difference in the percent of micronucleated reticulocytes detected between any of the MeNQ dose groups. The positive control group was significantly different ($P < 0.001$) from the negative control baseline, indicating that the test was correctly administered. See Appendix M for analysis of the data and the contract laboratory's report.

7.3.9 Sperm Analysis

No significant differences were noted in either sperm motility or number in the 14-day study. See Appendix N for the contributing scientist's report and analysis.

7.3.10 Pathology

The gross necropsy for the 14-day rats did not reveal many treatment or non-treatment related findings. A summary of the treatment related findings is summarized in Table 2.

Table 2: Treatment-Related Gross Findings

Dose	Rat Number	Sex	Finding
Control	14-0687	Male	Adhesion on liver
Control	14-0694	Female	Diffusely, mildly pale liver.
Control	14-0695	Female	Diffusely mildly pale liver
Control	14-0731	Female	Moderately pale liver
Control	14-0701	Female	Moderately pale liver
Control	14-0716	Female	Mildly pale liver (diffuse)
Control	14-0717	Female	Mildly pale liver (diffuse)
100 mg/kg	14-0663	Male	Enlarged cauda on right epididymis
100 mg/kg	14-0714	Female	Slightly prominent reticular pattern on liver.
210 mg/kg	14-0676	Male	Two white masses in caput of left epididymis
210 mg/kg	14-0748	Female	Minimally pale liver
415 mg/kg	14-0625	Male	Lesion in ileum of small intestine.
415 mg/kg	14-0710	Female	prominent cecal lymphatics
415 mg/kg	14-0711	Female	prominent cecal lymphatics; prominent left adrenal.
415 mg/kg	14-0720	Female	Diffusely mildly pale liver
415 mg/kg	14-0721	Female	Diffusely mildly pale liver
415 mg/kg	14-0728	Female	Diffusely mildly pale liver; 2 mm white lesion on left kidney
415 mg/kg	14-0729	Female	Diffusely mildly pale liver
415 mg/kg	14-0708	Female	Mildly pale liver (diffuse)
830 mg/kg	14-0670	Male	possible granuloma on left epididymis.
830 mg/kg	14-0713	Female	Slightly prominent reticular pattern on liver.
830 mg/kg	14-0736	Female	Diffusely mildly pale liver.
830 mg/kg	14-0698	Female	Mildly pale liver (diffuse)
830 mg/kg	14-0727	Female	Moderately pale liver
1250 mg/kg	14-0706	Female	Diffusely mildly pale liver
1250 mg/kg	14-0707	Female	Minimal palor on liver
1250 mg/kg	14-0725	Female	Mildly pale liver (diffuse)
1250 mg/kg	14-0739	Female	Mildly enlarged uterus

7.3.11 Histopathology

Histopathologic examination of a partial tissue list for the corn-oil control and high-dose (1250 mg/kg-d) animals and specific target organs (testes) for the next lowest dose group (830 mg/kg-d)

indicate that there are no significant treatment related findings in the any of the observed organs (brain, lung, heart, kidney, adrenal gland, liver, spleen and gonads (testes, epididymis, ovaries, uterus and cervix) due to administration of MeNQ in this study. Vacuolated Sertoli cells were noted in a 4 out of 10 males in the 1250 mg/kg-d dose group; however the incidence was not different from controls, with 1 out of 9 corn-oil controls showing a similar response. Eight out of 10 female lungs showed evidence of alveolar hemorrhage, but this was also found to be related to treatment. Three out of 10 females in the corn-oil control group also had alveolar hemorrhage. See Appendix L for the pathology report.

8 Discussion

The purpose of this study was to provide acute and subacute oral toxicity data on MeNQ. One previous study had assessed the acute oral toxicity of the compound at doses up to 1000 mg/kg with no effect on the animals [2]. This current study conducted acute testing at dosages up to 2000 mg/kg without any overt toxicity. Subacute (14 day) testing was commenced at dosages up to 1,250 mg/kg-d, again without any preclinical or histopathological toxicity. While in the female rats, mean lymphocyte proportions were increased in the three highest dose groups, this is likely not as a result of compound exposure. but is more likely to be attributed to an irritation and inflammatory response as a result of the oral gavage procedure and by the dosing compound over the 14-day exposure period. Sperm analysis and the micronucleus assay were also negative for effects at all dosages. Two different lesions were noted during histopathological evaluation, but neither were found to be related to treatment. Regarding the vacuolated Sertoli cells, there is evidence that MeNQ may be a factor in causing similar effects in mice treated with neonicotinoids such as clothiandin [4]. MeNQ is a potential metabolite of various neonicotinoids including clothiandin. However, variation was high for this observation and treated animals were not different from controls; greater numbers may be necessary to determine if MeNQ does affect Sertoli cells in rats. In female rats, alveolar hemorrhage was noted in a large number of the high dose animals; however, this was attributed to a perimortem event by the pathologist, potentially the intracardiac stick. The weight of evidence of these data suggests that MeNQ is not toxic at high oral exposures (1250 mg/kg-d) for a period of up to 14 days. Additional testing on this compound has included skin and eye irritation, skin sensitization, mutagenicity and metabolic products of MeNQ [2]. All tests conducted in this study were negative except for the eye irritation, where mild conjuctival irritation was noted and in the guinea pig maximization test, where one guinea pig out of an unspecified number showed a sensitizing reaction. Additionally, MeNQ is potentially metabolized to n-methyl-nitro-nitrosoguanidine, a known carcinogen; however this metabolite was not detected in the study, nor was there any evidence of systemic *in vivo* clastogenicity. Thus, by the tests already performed and with the additional data provided in this current study, it can be concluded that MeNQ is limited in its toxicity. The longest period of exposure for toxicity testing of MeNQ is the 14-day study described in this report; it is possible that longer exposure to the compound may result in adverse effects.

9 Conclusions

Daily oral exposures of MeNQ to male and female rats at dosages of 1250 mg/kg-d in corn oil for 14 days did not induce any signs of toxicity. There were no changes in body mass, body mass gain, clinical chemistry, hematology, organ weights or weight ratios, incidence of micronucleated reticulocytes, or effects on sperm motility or number at any of the dosages. There were no overt

clinical signs in the acute or subacute dosing periods. Microscopic analysis of the tissues revealed no significant effects at the highest exposure level.

Under the study conditions, oral administration of a single dose of MeNQ up to 2000 mg/kg or dosages up to 1250 mg/kg-d MeNQ for 14 days did not produce any toxic effects.

10 Point of Contact

Questions pertaining to this report should be referred to Emily N. Reinke, Ph.D. at DSN 584-3980, commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-aphc.mbx.tox-info@mail.mil.

Toxicology Study No. S.0024883, July-September 2014

Prepared By:

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19 Apr. 2016
Date

Approved By:

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4/19/16
Date

Mark S. Johnson, Ph.D., D.A.B.T
Portfolio Director, Toxicology

4/20/16
Date

APPENDIX A
REFERENCES

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APPENDIX B

QUALITY ASSURANCE STATEMENT

FOR: Toxicology Study No. S.0024883, Protocol No. 30-14-07-01, entitled "Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)," the following critical phases were inspected/audited by the Quality Systems and Regulatory Compliance (QSARC) Office:

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol Good Laboratory Practice Standards and Animal Care Review	06/05/2014	06/06/2014

IN-LIFE PHASE OF THE STUDY – ACUTE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Acute Study (LD50) - Test System Facilities, Identification, Husbandry, Feed and Water & Enrichment	07/22/2014	08/06/2014
Acute Study (LD50) -Test Article Storage, Control, Mixing, Labeling & Administration via Oral Gavage	07/22/2014	08/06/2014
Study Personnel Qualifications and Training Records Review	08/04/2014	08/06/2014
Analytical Chemistry Support - Dosing Solution Concentration Verification Procedures and Data Review	08/06/2014	08/06/2014
Acute Study (LD50) - Animal Euthanasia, Necropsy & Gross Macroscopic Pathology Exam Procedures	08/07/2014	08/15/2014
Acute Study (LD50) - Post Mortem Procedure Good Documentation Practices	08/07/2014	08/15/2014
Acute Study (LD50) - Sub-study Endpoint Criteria Compliance	08/07/2014	08/15/2014

IN-LIFE PHASE OF THE STUDY – SUBACUTE (14 DAY STUDY)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Subacute (14-day) study - Maintenance and Calibration of Equipment & Test Article Control Procedures	08/15/2014	08/27/2014
Subacute (14 Day) Study - Test Substance Preparation, Labeling and Administration via Oral Gavage	08/15/2014	08/27/2014
Subacute (14 Day) Study – Test System Facilities, Identification, Husbandry, & Feed and Water Supply	08/15/2014	08/27/2014
Subacute (14 Day) Study Pre-and Post-procedural Provisions and Good Documentation Procedures	08/15/2014	08/27/2014
Subacute Study - Males - Pre-Procedures, Anesthesia, and Blood Collection Procedures	08/15/2013	08/27/2014
Subacute Study - Males - Epididymis Dissection, Sperm Collection and Analysis Procedures	08/28/2014	09/4/2014
Subacute Study - Males - Euthanasia, Necropsy, Organ Collection, and Tissue Preservation Procedures	08/28/2014	09/4/2014
Subacute Study - Males - Blood Transfer, Clinical Chemistry and Hematology Procedures	08/28/2014	09/4/2014

APPENDIX B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. S.0024883, Protocol No. 30-14-07-01, entitled "Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)," the following critical phases were inspected/audited by the Quality Systems and Regulatory Compliance (QSARC) Office:

IN-LIFE PHASE OF THE STUDY – SUBACUTE (14 DAY STUDY) (continued)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Subacute Study - Females - Euthanasia, Necropsy, and Organ Collection Procedures	09/04/2014	09/12/2014
Subacute Study - Females - Biosample Collection and Analysis and Good Documentation Procedures	09/04/2014	09/12/2014
Subacute Study - Females - Necropsy Study Personnel Qualifications and Training Records Review	09/04/2014	09/12/2014
Final Study Endpoint Criteria Compliance	09/04/2014	09/12/2014

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Pathology Contributing Scientist Inspection-Interim Pathology Report GLP Standard Regulation Review	9/30/2015	10/07/2015
Pathology Contributing Scientist Inspection-QA audit of statistician's report and Excel Entered Data	10/01/2015	10/07/2015
Pathology Contributing Scientist Inspection- Final Pathology Report GLP Standard Regulation Review	10/06/2015	10/07/2015
Pathology Contributing Scientist Inspection - Final Study Raw Data GLP Standard Regulation Review	10/06/2015	10/07/2015
Contributing Scientist Report Review - Immunotoxicity of NTO in F1 Male & Female Rats Report review	01/13/2016	01/13/2016
Final Study Raw Data Good Laboratory Practice Quality Assurance Review	01/29/2016	02/02/2016
Final Study Report Good Laboratory Practice Quality Assurance Review	01/29/2016	02/02/2016

Note 1 All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2 In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QSARC Standing Operating Procedures.

Note 3 This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver

Michael P. Kefauver
Quality Assurance Specialist, QSARC

19 APR 2016

Date

APPENDIX C
ARCHIVES AND STUDY PERSONNEL

C-1. Archives

a. All raw data, documentation, records, protocol, maintenance and calibration logs, animal room log books, and a copy of the final report generated as a result of this study will be archived in the storage facilities of the Toxicology Portfolio, APHC (Prov) for a minimum of ten (10) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

b. Animal Records on animal receipt, diet, and facility environmental parameters will be archived by the Veterinary Medicine Division, Quality Systems and Regulatory Compliance Office, for a minimum of ten (10) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

c. The present study use the toxicology study number: S.0024883 and the protocol number 30-14-07-01 for all filings.

d. The protocol, physical raw data, summary data, and the final report pertaining to this study will be physically maintained within Building E-2100, APHC (Prov). These data may be scanned to a computer disk. Scanned study files and electronic necropsy files will be stored electronically on a CD in the study files and in the necropsy folder in the Toxicology shared network drive.

e. Archived SOPs will be maintained in Master Control.

f. Some ancillary records pertaining to this study, such as instrument maintenance logs, animal room observation logs, etc., will not be archived until those logbooks have been completed. Once completed, they will be archived in Room 1026 of building E-2100.

g. Animal Records Storage is the same as Animal Records.

h. Histologial samples will be maintained in the TOX portfolio cage in building E-5158, which is environmentally controlled.

i. Archivist: Martha Thompson, Toxicology Portfolio

C-2. Personnel

a. Management: Mark Johnson, Ph.D., DABT, Director of Toxicology; Michael J. Quinn, Ph.D., Program Manager, Health Effects Research Program (HERP).

b. Study Director: Emily N. Reinke, Ph.D., Biologist, HERP.

c. Quality Assurance: Michael P. Kefauver, Chemist; Thomas Sherwood, Ph.D., CPT MS, Quality Systems and Regulatory Compliance (QSARC).

d. Veterinary Medicine and Animal Care: Mary Beth Sprangel, DVM, MAJ, VC, Robert Sunderland III, Rebecca Kilby, Felicia Thomas, Brandin L. Versteegh, SPC, Veterinary Medicine Office, QSARC.

e. Necropsies: Erica E. Carroll, DVM, LTC VC, Alicia Shiflett, Histotechnician, Pathology Division, Toxicology.

f. In-Life Support: Emily N. Reinke, Ph.D., Biologist, HERP, Lee C.B. Crouse, Biologist, TEP, Michael J. Quinn, Ph.D., Biologist, HERP.

g. Pathology Laboratory Coordinator: Alicia Shiflett, Histotechnician, Pathology Division, Toxicology.

APPENDIX D
ANALYTICAL CHEMISTRY

Protocol No. 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table D-1
Summary of Acute Nominal Concentration

Nominal Concentration	7/29/2014
200 mg/ml	190

Table D-2
30-Day Sample Stability Data

Nominal Concentration	Sample Date				
	7/17/2014	7/23/2014	7/30/2014	8/6/2014	8/13/2014
10 mg/ml	9.9	9.4	9.3	9.2	9.2

Table D-3
Dosing Suspension Concentration Analysis

Sample Date	Nominal Concentrations of MeNQ in Corn Oil				
	14.28 mg/ml	30 mg/ml	59.28 mg/ml	118.57 mg/ml	178.57 mg/ml
8/11/2014	13.7	28	53.4	104.4	162.1
8/21/2014	13.1	28.7	51	102	140

Table D-4
Actual Dosages Delivered to Male Rats during 14-day study

Period		Nominal Dose of MeNQ in Corn Oil				
		100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Day 0	Mean	96.04	195.47	377.89	733.88	1115.72
	S.D.	1.73	2.32	5.31	8.44	36.81
	Range	93.8-98.0	192.2-199.0	367.5-382.5	724.1-744.9	1107.6-1138.8
	N	10	10	10	10	10
Day 1	Mean	95.90	195.45	375.82	730.84	1145.12
	S.D.	2.34	2.55	9.58	12.33	12.17
	Range	93.1-101.7	191.7-198.8	361.0-390.9	706.1-746.2	1112.6-1156.0
	N	10	10	10	10	10
Day 3	Mean	95.45	197.07	375.61	727.88	1141.45
	S.D.	1.41	2.63	3.98	6.42	10.58
	Range	94.1-98.8	192.1-200.5	368.3-380.0	716.8-742.5	1122.0-1152.4
	N	10	10	10	10	10
Day 7	Mean	96.35	196.50	375.88	732.66	1122.42
	S.D.	1.01	2.06	18.76	7.94	7.80
	Range	94.6-97.8	194.0-200.0	370.2-413.7	718.4-744.8	1113.3-1140.9
	N	10	10	10	10	10

Table D-5
Actual Dosages Delivered to Female Rats during 14-Day Study

Period		Nominal Dose of MeNQ in Corn Oil				
		100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Day 0	Mean	95.60	197.90	373.98	732.08	1142.74
	S.D.	1.89	3.39	8.57	18.09	18.42
	Range	94.0-98.6	190.8-201.0	362.9-386.2	708.6-754.7	1105.6-1164.5
	N	10	10	10	10	10
Day 1	Mean	95.63	193.36	374.61	728.19	1133.45
	S.D.	2.09	3.36	7.19	12.10	24.29
	Range	93.0-98.9	190.1-200.8	362.1-382.6	713.5-746.3	1100.4-1169.3
	N	10	10	10	10	10
Day 3	Mean	93.84	194.89	373.22	726.86	1121.35
	S.D.	3.29	3.37	12.80	14.22	21.71
	Range	89.5-98.0	189.6-200.4	350.0-399.6	708.0-748.7	1098.1-1159.0
	N	10	10	10	10	10
Day 7	Mean	91.64	199.02	359.18	710.62	988.12
	S.D.	1.65	1.89	6.78	15.00	14.49
	Range	89.1-94.4	197.1-201.7	347.2-361.6	692.4-733.2	964.3-1007.2
	N	10	10	10	10	10

APPENDIX E
SEQUENTIAL STAGE-WISE PROBIT

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table E-1

Protocol No.: 30-14-07-01 Date: 7-24-14 Stage 2 Dosing

Chemical Substance: MeNQ

Route: Oral **Species:** Sprague-Dawley Rat **Sex:** Male/Female

Concentration of Test Substance: 20 mg/ml[A], 200 mg/ml[B]

Diluent: Corn Oil

Animal Number	Sex	Dosing Stage	Weight kg	Nominal Dose mg/kg	Volume mL	Exposure Day Clinical Signs S*-sign	Exposure Day Morbidity/Mortality
14-0551	M	1	0.2304	61	0.7[A]	N	No
14-0552	M	1	0.2252	195	2.2[A]	N	No
14-0553	M	1	0.2372	625	0.74[B]	N	No
14-0554	M	1	0.2282	2000	2.3[B]	N	No
14-0581	F	1	0.173	61	0.5[A]	N	No
14-0582	F	1	0.1806	195	1.75[A]	N	No
14-0583	F	1	0.1661	625	0.52[B]	N	No
14-0584	F	1	0.1896	2000	1.9[B]	N	No
14-0555	M	2	0.2561	2000	2.561[B]	N	No
14-0556	M	2	0.2392	2000	2.4[B]	S1	No
14-0557	M	2	0.241	2000	2.4[B]	N	No
14-0558	M	2	0.2422	2000	2.4[B]	S1	No
14-0585	F	2	0.1997	2000	1.99[B]	N	No
14-0586	F	2	0.191	2000	1.91[B]	N	No
14-0587	F	2	0.189	2000	1.89[B]	N	No
14-0588	F	2	0.1825	2000	1.83[B]	N	No

* Signs : N - Normal S1 – Diarrhea

Doses were based on nominal concentrations of test suspensions.

Study Conclusions: Estimated LD_{50} of > 2000 mg/kg

APPENDIX F
SUMMARY OF 14-DAY CLINICAL OBSERVATIONS AND INDIVIDUAL DATA

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table F-1
14-day Clinical Observation Summary
Male Rats

Observation	Corn Oil Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Integumentary Signs						
Alopecia	1/10				1/10	
Barbering						1/10
Scab			1/10			
Respiration						
Congested breathing	1/10					
Mortality						
Early Sacrifice	1/10					
Terminal Sacrifice	9/10	10/10	10/10	10/10	10/10	10/10

Table F-2
14-day Clinical Observation Summary
Female Rats

Observation	Corn Oil Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Integumentary Signs						
Alopecia		1/10				
Skeletal Signs						
Abnormal Gait		1/10				
Mortality						
Terminal Sacrifice	10/10	10/10	10/10	10/10	10/10	10/10

Table F-3
14-Day Individual Clinical Observations
Male Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
Corn Oil Control	14-0639	Appears Normal			0	14
	14-0640	Appears Normal			0	14
	14-0687	Appears Normal			0	14
	14-0688	Alopecia	Face		1	2
	14-0635	Appears Normal			0	14
	14-0636	Punctured Esophagus		Early sacrifice	6	6
	14-0673	Appears Normal			0	14
	14-0674	Appears Normal			0	14
	14-0657	Appears Normal			0	14
	14-0658	Appears Normal			0	14
100 mg/kg	14-0645	Appears Normal			0	14
	14-0646	Appears Normal			0	14
	14-0663	Appears Normal			0	14
	14-0664	Appears Normal			0	14
	14-0653	Appears Normal			0	14
	14-0654	Appears Normal			0	14
	14-0641	Appears Normal			0	14
	14-0642	Appears Normal			0	14
	14-0677	Appears Normal			0	14
	14-0678	Appears Normal			0	14
210 mg/kg	14-0629	Appears Normal			0	14
	14-0630	Appears Normal			0	14
	14-0675	Appears Normal			0	14
	14-0676	Appears Normal			0	14
	14-0649	Appears Normal			0	14
	14-0650	Appears Normal			0	14
	14-0683	Scab	Back		6	14
	14-0684	Appears Normal			0	14
	14-0665	Appears Normal			0	14
	14-0666	Appears Normal			0	14
415 mg/kg	14-0625	Appears Normal			0	14
	14-0626	Appears Normal			0	14
	14-0667	Appears Normal			0	14
	14-0668	Appears Normal			0	14
	14-0633	Appears Normal			0	14
	14-0634	Appears Normal			0	14
	14-0681	Appears Normal			0	14
	14-0682	Appears Normal			0	14
	14-0643	Appears Normal			0	14
	14-0644	Appears Normal			0	14
830 mg/kg	14-0679	Appears Normal			0	14
	14-0680	Appears Normal			0	14
	14-0655	Appears Normal			0	14
	14-0656	Alopecia	Hind Legs		4	14
	14-0661	Appears Normal			0	14
	14-0662	Appears Normal			0	14
	14-0669	Appears Normal			0	14
	14-0670	Appears Normal			0	14
	14-0685	Appears Normal			0	14
	14-0686	Appears Normal			0	14

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
1250 mg/kg	14-0637	Appears Normal			0	14
	14-0638	Appears Normal			0	14
	14-0627	Appears Normal			0	14
	14-0628	Barbering	Forepaws		7	14
	14-0671	Appears Normal			0	14
	14-0672	Appears Normal			0	14
	14-0631	Appears Normal			0	14
	14-0632	Appears Normal			0	14
	14-0651	Appears Normal			0	14
	14-0652	Appears Normal			0	14

Table F-4
14-Day Clinical Observations
Female Rats

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
Corn Oil Control	14-0694	Appears Normal			0	14
	14-0695	Appears Normal			0	14
	14-0730	Appears Normal			0	14
	14-0731	Appears Normal			0	14
	14-0700	Appears Normal			0	14
	14-0701	Appears Normal			0	14
	14-0732	Appears Normal			0	14
	14-0733	Appears Normal			0	14
	14-0716	Appears Normal			0	14
	14-0717	Appears Normal			0	14
100 mg/kg	14-0714	Appears Normal			0	14
	14-0715	Appears Normal			0	14
	14-0742	Appears Normal			0	14
	14-0743	Appears Normal			0	14
	14-0692	Appears Normal			0	14
	14-0693	Appears Normal			0	14
	14-0702	Appears Normal			0	14
	14-0703	Abnormal gait	Hind legs	Mild	0	14
	14-0740	Alopecia	Right shoulder		0	6
	14-0741	Appears Normal			0	14
210 mg/kg	14-0690	Appears Normal			0	14
	14-0691	Appears Normal			0	14
	14-0746	Appears Normal			0	14
	14-0747	Appears Normal			0	14
	14-0718	Appears Normal			0	14
	14-0719	Appears Normal			0	14
	14-0748	Appears Normal			0	14
	14-0749	Appears Normal			0	14
	14-0744	Appears Normal			0	14
	14-0745	Appears Normal			0	14
415 mg/kg	14-0710	Appears Normal			0	14
	14-0711	Appears Normal			0	14
	14-0722	Appears Normal			0	14
	14-0723	Appears Normal			0	14
	14-0720	Appears Normal			0	14
	14-0721	Appears Normal			0	14
	14-0728	Appears Normal			0	14
	14-0729	Appears Normal			0	14
	14-0708	Appears Normal			0	14
	14-0709	Appears Normal			0	14
830 mg/kg	14-0712	Appears Normal			0	14
	14-0713	Appears Normal			0	14
	14-0704	Appears Normal			0	14
	14-0705	Appears Normal			0	14
	14-0736	Appears Normal			0	14
	14-0737	Appears Normal			0	14
	14-0698	Appears Normal			0	14
	14-0699	Appears Normal			0	14
	14-0726	Appears Normal			0	14
	14-0727	Appears Normal			0	14

Group	Animal ID	Observation	Location (if applicable)	Severity (if applicable)	First Day Observed	Last Day Observed
1250 mg/kg	14-0706	Appears Normal			0	14
	14-0707	Appears Normal			0	14
	14-0696	Appears Normal			0	14
	14-0697	Appears Normal			0	14
	14-0734	Appears Normal			0	14
	14-0735	Appears Normal			0	14
	14-0724	Appears Normal			0	14
	14-0725	Appears Normal			0	14
	14-0738	Appears Normal			0	14
	14-0739	Appears Normal			0	14

APPENDIX G
SUMMARY OF 14-DAY BODY WEIGHTS AND INDIVIDUAL DATA

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

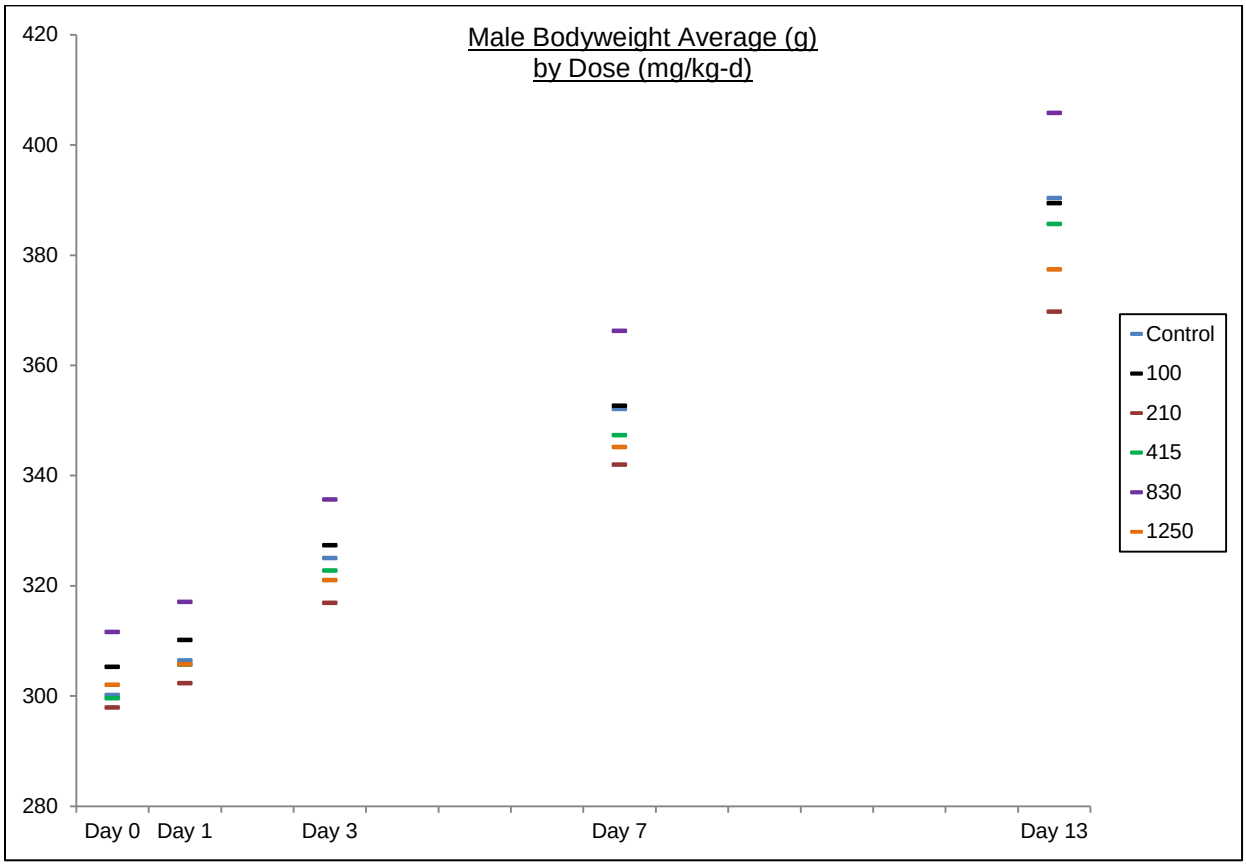

Table G-1
Summary of 14-Day Body Weights (grams)
Male Rats

Period		Corn Oil Control	MeNQ in Corn Oil 100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Day 0	Mean	300.18	305.27	297.92	299.57	311.61	302.02
	S.D.	10.43	17.40	13.69	13.64	11.24	11.31
	N	10	10	10	10	10	10
Day 1	Mean	306.44	310.18	302.30	305.66	317.05	305.72
	S.D.	12.71	17.32	15.80	15.87	12.54	15.84
	N	10	10	10	10	10	10
Day 3	Mean	325.03	327.36	316.87	322.74	335.66	321.01
	S.D.	13.89	20.19	16.71	17.78	15.04	17.96
	N	10	10	10	10	10	10
Day 7	Mean	352.06	352.63	341.98	347.30	366.25	345.17
	S.D.	18.59	22.96	15.79	20.44	20.36	19.79
	N	9	10	10	10	10	10
Day 13	Mean	390.34	389.41	369.70	385.65	405.76	377.41
	S.D.	29.62	32.60	29.93	25.87	29.37	24.80
	N	9	10	10	10	10	10

Table G-2
14-Day Individual Body Weights (grams)
Male Rats

Dose Group	Animal ID	Day 0	Day 1	day 3	Day 7	Day 13
Corn Oil Control	14-0635	307.40	319.90	341.60	371.90	427.30
	14-0636	313.60	318.10	338.90	ND	ND
	14-0639	287.90	292.00	312.70	347.00	387.80
	14-0640	298.40	311.40	334.50	385.80	446.10
	14-0657	302.20	310.00	319.80	343.20	371.20
	14-0658	314.30	323.80	340.20	362.70	397.10
	14-0673	279.50	283.50	294.70	316.30	337.60
	14-0674	306.30	312.30	326.20	347.40	381.70
	14-0687	295.60	296.70	319.10	344.80	384.60
	14-0688	296.60	296.70	322.60	349.40	379.70
	Mean	**300.18**	**306.44**	**325.03**	**352.06**	**390.34**
	SD	**10.43**	**12.71**	**13.89**	**18.59**	**29.62**
100 mg/kg	14-0641	325.40	324.80	342.50	371.80	415.80
	14-0642	318.20	323.80	340.80	369.00	403.60
	14-0645	286.80	290.20	305.90	331.20	355.50
	14-0646	294.50	298.50	316.20	340.10	380.40
	14-0653	294.80	300.90	318.70	342.80	386.90
	14-0654	294.10	300.80	316.90	336.10	359.50
	14-0663	318.80	321.20	344.60	380.20	430.50
	14-0664	277.60	283.00	291.20	312.50	330.60
	14-0677	321.00	328.90	348.00	366.10	418.10
	14-0678	321.50	329.70	348.80	376.50	413.20
	Mean	**305.27**	**310.18**	**327.36**	**352.63**	**389.41**
	SD	**17.40**	**17.32**	**20.19**	**22.96**	**32.60**
210 mg/kg	14-0629	309.60	314.30	326.80	350.00	386.40
	14-0630	291.30	296.50	311.40	343.30	376.30
	14-0649	285.50	290.10	307.20	331.90	331.90
	14-0650	283.60	286.90	301.80	324.40	324.40
	14-0665	313.80	324.00	336.60	368.30	423.50
	14-0666	318.20	321.30	335.20	355.50	388.10
	14-0675	290.20	287.20	302.40	325.50	348.10
	14-0676	285.00	286.10	294.80	328.50	361.20
	14-0683	311.90	320.30	341.50	360.40	391.40
	14-0684	290.10	296.30	311.00	332.00	365.70
	Mean	**297.92**	**302.30**	**316.87**	**341.98**	**369.70**
	SD	**13.69**	**15.80**	**16.71**	**15.79**	**29.93**
415 mg/kg	14-0625	297.60	310.60	323.20	355.80	387.30
	14-0626	293.20	298.60	317.50	350.30	389.00
	14-0633	290.60	297.20	310.80	326.60	365.00
	14-0634	302.50	308.80	324.40	346.20	393.10
	14-0643	295.00	300.40	319.00	335.60	374.40
	14-0644	337.10	347.80	370.80	398.20	449.40
	14-0667	293.30	293.20	310.60	337.70	374.10
	14-0668	293.70	294.50	311.20	329.40	355.60
	14-0681	299.20	305.00	325.00	353.90	396.50
	14-0682	293.50	300.50	314.90	339.30	372.10
	Mean	**299.57**	**305.66**	**322.74**	**347.30**	**385.65**
	SD	**13.64**	**15.87**	**17.78**	**20.44**	**25.87**

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Dose Group	Animal ID	Day 0	Day 1	day 3	Day 7	Day 13
	14-0655	313.30	315.00	335.00	372.70	414.80
830 mg/kg	14-0656	326.20	336.80	360.70	408.90	467.90
	14-0661	308.40	317.90	329.90	363.30	400.10
	14-0662	310.00	311.00	329.80	355.50	390.40
	14-0669	295.30	299.80	317.80	336.40	355.80
	14-0670	320.70	322.50	345.20	371.50	413.60
	14-0679	296.30	305.50	313.80	340.50	374.00
	14-0680	300.10	300.90	323.40	358.80	406.10
	14-0685	317.20	325.30	343.90	365.80	400.50
	14-0686	328.60	335.80	357.10	389.10	434.40
	Mean	**311.61**	**317.05**	**335.66**	**366.25**	**405.76**
	SD	**11.24**	**12.54**	**15.04**	**20.36**	**29.37**
	14-0627	302.30	312.90	327.30	364.00	408.40
1250 mg/kg	14-0628	307.60	312.30	329.00	348.20	373.00
	14-0631	306.80	309.90	326.00	345.10	365.00
	14-0632	292.70	291.40	303.40	317.70	340.80
	14-0637	287.70	280.50	295.40	326.80	370.30
	14-0638	285.30	284.00	295.70	319.70	350.60
	14-0651	302.40	309.50	322.10	346.80	367.90
	14-0652	327.40	338.00	358.90	387.50	429.60
	14-0671	305.10	310.20	325.00	348.00	384.90
	14-0672	302.90	308.50	327.30	347.90	383.60
	Mean	**302.02**	**305.72**	**321.01**	**345.17**	**377.41**
	SD	**11.31**	**15.84**	**17.96**	**19.79**	**24.80**

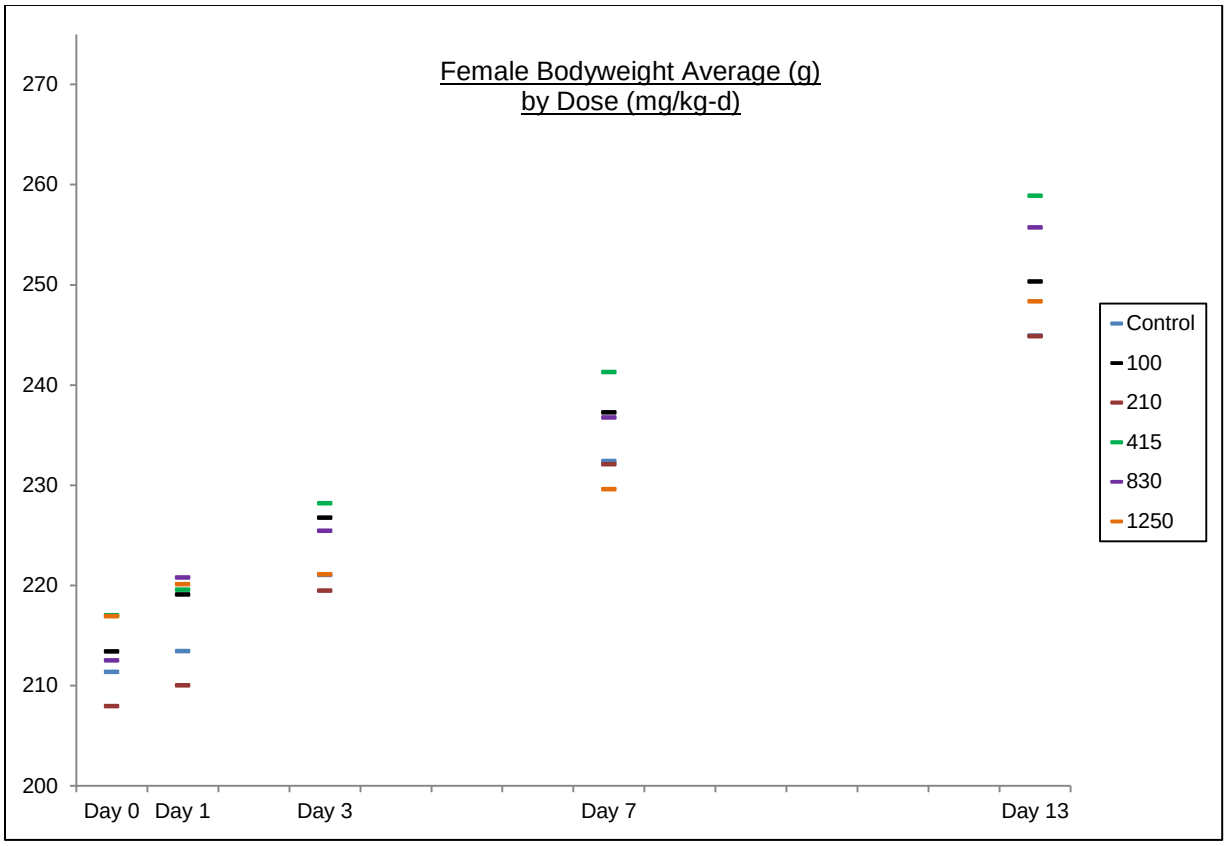
Female Bodyweight Average (g)
by Dose (mg/kg-d)
270
260
250
240
230
220
210
200
Day 0
Day 1
Day 3
Day 7
Day 13
Control
100
210
415
830
1250

Table G-3
Summary of 14-Day Body Weights (grams)
Female Rats

Period		Corn Oil Control	MeNQ in Corn Oil				
			100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Day 0	Mean	211.36	213.42	207.96	217.03	212.52	216.93
	S.D.	11.71	6.98	8.32	15.18	11.84	12.91
	N	10	10	10	10	10	10
Day 1	Mean	213.43	219.08	210.02	219.56	220.77	220.11
	S.D.	13.12	10.16	10.70	15.14	11.05	12.33
	N	10.00	10.00	10.00	10.00	10.00	10.00
Day 3	Mean	221.05	226.74	219.48	238.19	225.43	221.10
	S.D.	13.24	12.08	13.06	34.32	11.42	15.10
	N	10	10	10	10	10	10
Day 7	Mean	232.41	237.26	232.10	241.27	236.76	229.60
	S.D.	17.67	14.15	18.57	19.08	14.56	18.05
	N	10	10	10	10	10	10
Day 13	Mean	244.94	250.32	244.86	258.89	255.72	248.33
	S.D.	19.46	18.56	25.32	21.36	15.60	18.00
	N	10	10	10	10	10	10

Table G-4
14-Day Individual Body Weights (grams)
Female Rats

Dose Group	Animal ID	Day 0	Day 1	day 3	Day 7	Day 13
Corn Oil Control	14-0694	202.10	198.80	214.10	230.80	233.50
	14-0695	208.90	206.20	215.70	220.70	231.40
	14-0700	231.40	237.00	239.70	342.70	250.70
	14-0701	194.40	195.90	203.50	210.10	217.50
	14-0716	213.00	213.90	214.30	218.80	236.20
	14-0717	205.60	208.90	204.70	212.90	235.80
	14-0730	231.10	231.20	240.30	256.10	271.40
	14-0731	210.80	220.70	233.60	262.90	282.60
	14-0732	206.30	213.70	221.50	233.80	249.00
	14-0733	210.00	208.00	223.10	235.30	241.30
	Mean	**211.36**	**213.43**	**221.05**	**232.41**	**244.94**
	SD	**11.71**	**13.12**	**13.24**	**17.67**	**19.46**
100 mg/kg	14-0692	203.90	204.80	212.10	220.40	232.10
	14-0693	215.40	228.10	238.10	254.20	267.70
	14-0702	211.80	216.10	216.40	224.70	243.90
	14-0703	205.60	214.60	219.50	227.50	236.30
	14-0714	222.70	229.90	234.90	247.30	263.30
	14-0715	204.20	206.10	209.60	218.20	221.60
	14-0740	222.30	235.80	246.80	258.10	284.20
	14-0741	214.40	220.90	229.60	236.00	248.90
	14-0742	218.50	212.80	227.30	240.80	246.50
	14-0743	215.40	221.70	233.10	245.40	258.70
	Mean	**213.42**	**219.08**	**226.74**	**237.26**	**250.32**
	SD	**6.98**	**10.16**	**12.08**	**14.15**	**18.56**
210 mg/kg	14-0690	205.40	200.90	217.70	230.60	229.80
	14-0691	201.80	204.80	200.50	203.90	219.80
	14-0718	194.90	201.10	206.70	215.30	226.20
	14-0719	214.50	207.10	218.10	220.20	227.00
	14-0744	224.40	231.80	245.30	270.40	297.90
	14-0745	199.10	209.20	214.80	228.80	237.80
	14-0746	209.50	202.90	218.00	228.60	234.50
	14-0747	208.90	201.10	212.00	231.10	237.30
	14-0748	210.70	220.40	229.80	245.30	264.70
	14-0749	210.40	220.90	231.90	246.80	273.60
	Mean	**207.96**	**210.02**	**219.48**	**232.10**	**244.86**
	SD	**8.32**	**10.70**	**13.06**	**18.57**	**25.32**
415 mg/kg	14-0708	236.10	242.40	247.70	264.60	298.90
	14-0709	240.40	238.90	250.10	265.40	279.70
	14-0710	219.10	226.80	226.10	237.00	259.00
	14-0711	194.50	196.50	204.20	200.40	222.50
	14-0720	217.60	223.30	237.90	254.60	271.90
	14-0721	207.40	212.00	218.70	229.50	237.40
	14-0722	201.00	197.10	215.00	238.30	250.70
	14-0723	230.40	219.70	227.20	249.70	252.60
	14-0728	217.80	221.20	228.00	239.80	260.40
	14-0729	206.00	217.70	227.00	233.40	255.80
	Mean	**217.03**	**219.56**	**228.19**	**241.27**	**258.89**
	SD	**15.18**	**15.14**	**14.15**	**19.08**	**21.36**

Dose Group	Animal ID	Day 0	Day 1	day 3	Day 7	Day 13
	14-0698	227.30	231.50	223.10	232.80	262.70
830 mg/kg	14-0699	207.50	216.80	228.70	235.70	251.20
	14-0704	222.30	234.10	240.60	249.10	262.70
	14-0705	204.00	217.60	217.00	222.60	240.00
	14-0712	193.10	204.20	205.40	218.30	231.50
	14-0713	221.00	211.50	232.90	250.50	253.70
	14-0726	221.00	225.50	221.20	229.20	266.60
	14-0727	202.40	210.80	219.10	228.50	243.90
	14-0736	202.70	217.90	222.60	234.50	258.00
	14-0737	223.90	237.80	243.70	266.40	286.90
	Mean	**212.52**	**220.77**	**225.43**	**236.76**	**255.72**
	SD	**11.84**	**11.05**	**11.42**	**14.56**	**15.60**
	14-0696	214.00	219.90	227.30	243.80	268.30
1250 mg/kg	14-0697	190.60	191.50	191.90	196.50	208.00
	14-0706	226.80	224.90	238.70	250.20	256.00
	14-0707	208.80	214.40	206.30	211.20	238.00
	14-0724	238.90	241.30	234.60	245.40	272.20
	14-0725	223.40	225.70	220.40	223.40	246.50
	14-0734	214.00	221.80	232.10	236.90	246.00
	14-0735	210.40	219.40	215.70	227.40	255.90
	14-0738	224.60	223.10	234.20	246.80	252.50
	14-0739	217.80	219.10	209.80	214.40	239.90
	Mean	**216.93**	**220.11**	**221.10**	**229.60**	**248.33**
	SD	**12.91**	**12.33**	**15.10**	**18.05**	**18.00**

APPENDIX H
SUMMARY OF 14-DAY CLINICAL CHEMISTRY AND INDIVIDUAL DATA
Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table H-1
14-Day Clinical Chemistry Summary
Male Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
ALB	Mean	2.69	2.72	2.66	2.72	2.82	2.71
(g/dL)	S.D.	0.13	0.12	0.11	0.13	0.18	0.16
	N	9	10	10	10	10	10
ALKP	Mean	279.67	277.50	279.50	291.70	280.80	270.50
(U/L)	S.D.	45.86	46.28	62.63	56.09	40.84	57.77
	N	9	10	10	10	10	10
ALT	Mean	73.78	82.00	99.90	89.90	88.60	93.80
(U/L)	S.D.	20.84	12.76	19.50	8.77	17.19	31.06
	N	9	10	10	10	10	10
AST	Mean	106.00	90.20	95.60	97.00	97.70	96.90
(U/L)	S.D.	32.41	10.18	30.57	14.90	17.38	12.72
	N	9	10	10	10	10	10
BUN	Mean	13.22	15.60	12.90	12.50	12.50	13.20
(mg/dL)	S.D.	4.41	4.14	2.64	2.64	2.95	3.65
	N	9	10	10	10	10	10
CA	Mean	11.17	11.19	10.85	11.32	11.37	10.99
(mg/dL)	S.D.	0.49	0.39	0.44	0.24	0.37	0.47
	N	9	10	10	10	10	10
CHOL	Mean	73.33	76.00	71.10	74.30	82.60	75.80
(mg/dL)	S.D.	14.71	13.17	12.34	11.31	17.93	9.52
	N	9	10	10	10	10	10
CREA	Mean	0.68	0.71	0.70	0.74	0.73	0.68
(mg/dL)	S.D.	0.13	0.14	0.11	0.10	0.14	0.14
	N	9	10	10	10	10	10
GLOB	Mean	3.24	3.39	3.30	3.34	3.55[a]	3.33
(mg/dL)	S.D.	0.13	0.14	0.13	0.21	0.20	0.11
	N	9	10	10	10	10	10
GLU	Mean	172.89	167.40	119.80	133.90	140.30	145.60
(mg/dL)	S.D.	51.19	44.50	55.39	52.90	39.22	33.93
	N	9	10	10	10	10	10
PHOS	Mean	13.53	12.67	12.49	14.32	13.82	13.02
(mg/dL)	S.D.	0.91	1.34	1.31	1.53	2.01	2.13
	N	9	10	10	10	10	10
TP	Mean	5.94	6.09	5.96	6.09	6.35[b]	6.03
(g/dL)	S.D.	0.20	0.19	0.22	0.29	0.33	0.24
	N	9	10	10	10	10	10

		Corn Oil Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
TRIG	Mean	85.67	88.10	80.60	80.20	82.70	57.50
(mg/dL)	S.D.	44.55	25.12	28.44	23.19	29.07	15.36
	N	9	10	10	10	10	10
Na	Mean	151.11	151.20	150.10	150.80	150.50	149.60
(mmol/L)	S.D.	1.90	1.55	1.73	1.40	1.78	2.50
	N	9	10	10	10	10	10
K	Mean	8.23	8.21	8.42	9.47	9.65	8.73
(mmol/L)	S.D.	1.43	1.32	1.72	1.45	1.84	1.42
	N	9	10	10	10	10	10
Cl	Mean	104.78	103.80	104.60	103.90	104.20	103.10
(mmol/L)	S.D.	1.30	1.32	1.78	1.45	1.40	1.29
	N	9	10	10	10	10	10

a. Significantly different than the control, 210, 415, and 1250 mg/kg dose groups, P = 0.002
b. Significantly different than the control and 210 mg/kg dose groups, P = 0.01

Table H-2
14-Day Individual Clinical Chemistry
Male Rats

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Notes
	14-635	2.7	324	51	131	10	10.7	50	0.5	3.1	177	12.1	5.8	60	150	6.9	105	
Corn Oil	14-639	2.8	319	99	88	13	11.1	92	0.8	3.2	242	13.8	6.0	200	151	7.5	105	
Control	14-640	2.9	261	55	89	14	11.4	94	0.6	3.5	134	14.5	6.4	93	153	10.1	104	
	14-657	2.5	285	79	157	12	11.6	68	0.9	3.3	233	14.3	5.9	72	151	7.9	102	
	14-658	2.6	218	48	74	9	11.1	87	0.6	3.2	197	13.8	5.8	89	150	7.5	105	
	14-673	2.7	208	64	93	11	10.1	65	0.6	3.2	86	12.9	5.9	65	150	7.6	106	
	14-674	2.6	261	88	77	10	11.5	72	0.7	3.1	186	14.0	5.7	63	148	7.5	104	
	14-687	2.8	308	105	154	17	11.6	69	0.8	3.2	121	14.2	6.0	70	154	11.2	106	
	14-688	2.6	333	75	91	23	11.4	63	0.6	3.4	180	12.2	6.0	59	153	7.9	106	
	Mean	**2.69**	**279.67**	**73.78**	**106.00**	**13.22**	**11.17**	**73.33**	**0.68**	**3.24**	**172.89**	**13.53**	**5.94**	**85.67**	**151.11**	**8.23**	**104.78**	
	S.D.	**0.13**	**45.86**	**20.84**	**32.41**	**4.41**	**0.49**	**14.71**	**0.13**	**0.13**	**51.19**	**0.91**	**0.20**	**44.55**	**1.90**	**1.43**	**1.30**	
	14-641	2.6	345	103	97	11	11.0	95	0.5	3.4	213	12.5	5.9	109	148	6.8	104	
100 mg/kg	14-642	2.5	352	84	111	13	11.2	77	0.7	3.5	132	13.6	6.1	100	152	8.1	106	
	14-645	2.7	244	65	89	18	10.9	76	0.6	3.2	150	10.4	5.9	56	149	6.6	102	
	14-646	2.9	209	71	82	17	11.8	85	0.7	3.5	162	14.6	6.4	126	153	10.3	104	
	14-653	2.8	282	84	80	13	11.5	67	0.9	3.4	203	14.3	6.2	93	152	10.0	103	
	14-654	2.8	226	95	87	19	10.7	69	0.8	3.6	88	12.3	6.3	48	151	8.4	102	
	14-663	2.6	298	89	95	13	10.9	95	0.7	3.3	146	12.8	5.9	94	152	8.1	104	
	14-664	2.7	264	67	80	11	10.8	60	0.5	3.2	218	10.8	5.9	91	151	6.5	105	
	14-677	2.8	271	90	82	17	11.7	79	0.9	3.3	221	13.0	6.1	103	152	9.0	105	
	14-678	2.8	284	72	99	24	11.4	57	0.8	3.5	141	12.4	6.2	61	152	8.3	103	
	Mean	**2.72**	**277.50**	**82.00**	**90.20**	**15.60**	**11.19**	**76.00**	**0.71**	**3.39**	**167.40**	**12.67**	**6.09**	**88.10**	**151.20**	**8.21**	**103.80**	
	S.D.	**0.12**	**46.28**	**12.76**	**10.18**	**4.14**	**0.39**	**13.17**	**0.14**	**0.14**	**44.50**	**1.34**	**0.19**	**25.12**	**1.55**	**1.32**	**1.32**	
210 mg/kg	14-630	2.6	316	114	84	16	10.3	49	0.6	3.3	96	9.8	5.8	76	149	6.4	108	
	14-649	2.6	371	91	94	9	10.5	73	0.6	3.4	78	12.6	6.1	47	149	7.6	105	
	14-650	2.9	188	79	76	12	11.1	59	0.8	3.5	194	13.0	6.4	60	154	8.3	104	
	14-665	2.7	303	92	84	14	11.1	86	0.6	3.3	187	12.0	6.0	80	150	7.2	102	
	14-666	2.7	213	96	80	10	11.4	60	0.7	3.2	181	14.7	5.9	59	150	11.8	104	
	14-675	2.5	338	117	121	12	10.5	81	0.7	3.2	61	12.3	5.7	141	148	8.1	106	
	14-676	2.6	277	118	174	15	10.2	73	0.8	3.2	76	11.4	5.8	115	150	7.1	105	
	14-683	2.6	224	65	74	10	11.3	88	0.9	3.3	89	13.7	5.9	70	152	11.1	103	
	14-684	2.7	336	99	86	16	10.9	71	0.7	3.5	67	12.7	6.2	91	150	8.3	103	
	Mean	**2.66**	**279.50**	**99.90**	**95.60**	**12.90**	**10.85**	**71.10**	**0.70**	**3.30**	**119.80**	**12.49**	**5.96**	**80.60**	**150.10**	**8.42**	**104.60**	
	S.D.	**0.11**	**62.63**	**19.50**	**30.57**	**2.64**	**0.44**	**12.34**	**0.11**	**0.13**	**55.39**	**1.31**	**0.22**	**28.44**	**1.73**	**1.72**	**1.78**	
	14-625	2.7	357	102	101	10	11.2	91	0.7	3.5	98	15.0	6.2	131	152	8.2	104	
415 mg/kg	14-626	2.7	267	94	105	13	11.2	85	0.8	3.0	269	14.0	5.8	94	151	9.3	103	
	14-633	2.6	244	93	126	11	10.9	68	0.8	3.5	134	14.8	6.2	76	152	9.1	104	
	14-634	2.8	200	78	68	14	11.1	76	0.9	3.2	150	13.4	6.0	58	152	9.2	102	PHOS 1:1 dilution
	14-643	2.6	311	92	102	12	11.4	54	0.7	3.1	89	15.5	5.7	54	149	11.3	104	
	14-644	2.8	346	95	90	17	11.5	70	0.8	3.6	140	14.2	6.5	86	152	9.0	102	
	14-667	2.6	315	73	98	13	11.2	65	0.6	3.3	108	11.4	5.9	53	149	8.5	106	
	14-668	3.0	369	97	91	16	11.7	71	0.8	3.6	88	17.3	6.6	90	152	12.5	106	
	14-681	2.6	260	88	87	9	11.6	88	0.6	3.4	148	13.6	6.1	81	149	9.9	103	
	14-682	2.8	248	87	102	10	11.4	75	0.7	3.2	115	14.0	5.9	79	150	7.7	105	

	Mean	**2.72**	**291.70**	**89.90**	**97.00**	**12.50**	**11.32**	**74.30**	**0.74**	**3.34**	**133.90**	**14.32**	**6.09**	**80.20**	**150.80**	**9.47**	**103.90**	
	S.D.	**0.13**	**56.09**	**8.77**	**14.90**	**2.64**	**0.24**	**11.31**	**0.10**	**0.21**	**52.90**	**1.53**	**0.29**	**23.19**	**1.40**	**1.45**	**1.45**	

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Notes
	14-655	2.9	321	69	88	14	11.3	111	0.7	3.8	111	14.0	6.7	48	152	9.1	104	
830 mg/kg	14-656	3.0	301	77	108	13	11.3	84	0.7	3.7	172	14.6	6.7	129	152	9.2	105	
	14-661	2.8	320	96	136	11	11.7	70	0.9	3.3	165	17.7	6.1	73	150	11.7	103	
	14-662	3.0	219	100	76	13	11.4	66	0.9	3.7	82	15.0	6.7	91	152	11.9	103	
	14-669	2.9	273	105	107	18	11.5	96	0.7	3.8	139	12.9	6.6	111	153	8.2	102	PHOS 1:1 dilution
	14-670	2.8	262	111	96	16	11.2	90	0.6	3.4	123	13.0	6.2	91	149	7.4	105	
	14-679	2.4	294	63	95	8	10.7	74	0.5	3.3	195	10.3	5.7	48	148	7.2	104	
	14-680	2.8	335	81	104	10	11.4	86	0.6	3.6	170	12.4	6.4	68	150	9.7	105	
	14-685	2.9	219	107	79	11	12.1	50	0.9	3.4	163	15.4	6.2	114	151	12.4	104	PHOS 1:1 dilution
	14-686	2.7	264	77	88	11	11.1	99	0.8	3.5	83	12.9	6.2	54	148	9.7	107	
	Mean	**2.82**	**280.80**	**88.60**	**97.70**	**12.50**	**11.37**	**82.60**	**0.73**	**3.55**	**140.30**	**13.82**	**6.35**	**82.70**	**150.50**	**9.65**	**104.20**	
	S.D.	**0.18**	**40.84**	**17.19**	**17.38**	**2.95**	**0.37**	**17.93**	**0.14**	**0.20**	**39.22**	**2.01**	**0.33**	**29.07**	**1.78**	**1.84**	**1.40**	
	14-627	2.9	287	66	85	8	11.6	71	0.8	3.5	191	16.5	6.4	60	154	9.4	103	
1250 mg/kg	14-628	2.8	259	105	108	17	11.4	91	0.7	3.3	131	15.2	6.1	49	150	10.0	102	
	14-631	2.9	269	108	87	17	10.4	69	0.6	3.4	134	12.7	6.3	34	147	7.2	104	PHOS 1:1 dilution
	14-632	2.8	374	169	126	17	11.5	81	0.9	3.4	111	13.8	6.1	61	151	9.7	104	
	14-637	2.4	357	72	95	16	10.2	73	0.5	3.1	176	11.2	5.6	51	146	7.4	104	
	14-638	2.6	254	107	100	12	10.9	89	0.6	3.3	204	10.9	5.9	53	152	8.5	103	
	14-651	2.8	252	90	82	15	11.4	78	0.8	3.4	114	15.6	6.2	60	149	11.6	104	
	14-652	2.6	217	68	97	11	10.8	78	0.8	3.3	144	12.3	5.8	46	147	8.2	100	
	14-671	2.6	183	77	94	11	10.9	61	0.6	3.3	143	11.2	5.9	91	151	7.7	103	
	14-672	2.7	253	76	95	8	10.8	67	0.5	3.3	108	10.8	6.0	70	149	7.6	104	
	Mean	**2.71**	**270.50**	**93.80**	**96.90**	**13.20**	**10.99**	**75.80**	**0.68**	**3.33**	**145.60**	**13.02**	**6.03**	**57.50**	**149.60**	**8.73**	**103.10**	
	SD	**0.81**	**85.68**	**29.02**	**38.47**	**3.74**	**3.33**	**22.26**	**0.23**	**1.01**	**63.67**	**3.96**	**1.83**	**33.99**	**47.16**	**2.46**	**32.30**	

Table H-3
14-Day Clinical Chemistry Summary
Female Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
ALB	Mean	3.10	3.25	3.14	3.23	3.15	3.37
(g/dL)	S.D.	0.45	0.21	0.25	0.18	0.19	0.21
	N	10	10	10	10	10	10
ALKP	Mean	144.00	158.60	179.40	174.00	157.60	169.10
(U/L)	S.D.	29.27	44.26	24.65	47.92	32.00	46.34
	N	10	10	10	10	10	10
ALT	Mean	75.80	81.40	77.80	81.90	86.20	82.30
(U/L)	S.D.	24.14	11.25	25.07	21.88	13.09	16.45
	N	10	10	10	10	10	10
AST	Mean	154.20	101.60	134.00	104.70	109.30	100.40
(U/L)	S.D.	143.10	26.17	97.09	34.08	37.92	15.22
	N	10	10	10	10	10	10
BUN	Mean	19.70	22.50	22.30	24.50	19.90	19.00[a]
(mg/dL)	S.D.	4.57	3.63	3.77	5.28	3.73	2.83
	N	10	10	10	10	10	10
CA	Mean	11.32	11.36	11.64	11.70	11.17	11.50
(mg/dL)	S.D.	0.43	0.54	0.60	0.60	0.53	0.52
	N	10	10	10	10	10	10
CHOL	Mean	63.30	72.20	74.10	70.30	70.00	69.30
(mg/dL)	S.D.	14.39	11.72	15.06	12.66	16.97	12.17
	N	10	10	10	10	10	10
CREA	Mean	0.68	0.79	0.75	0.77	0.68	0.67
(mg/dL)	S.D.	0.09	0.14	0.11	0.11	0.11	0.12
	N	10	10	10	10	10	10
GLOB	Mean	3.32	3.20	3.23	3.18	3.25	3.20
(mg/dL)	S.D.	0.28	0.31	0.14	0.23	0.11	0.25
	N	10	10	10	10	10	10
GLU	Mean	131.50	111.90	92.60	132.80	115.20	157.10
(mg/dL)	S.D.	52.97	52.67	21.25	73.79	40.28	75.31
	N	10	10	10	10	10	10
PHOS	Mean	13.91	14.24	14.76	14.63	13.99	13.92
(mg/dL)	S.D.	2.36	2.00	2.27	1.90	2.42	2.20
	N	10	10	10	10	10	10
TP	Mean	6.42	6.44	6.36	6.41	6.40	6.58
(g/dL)	S.D.	0.34	0.45	0.35	0.36	0.25	0.33
	N	10	10	10	10	10	10
TRIG	Mean	40.20	45.30	42.80	41.90	28.40[b]	34.10
(mg/dL)	S.D.	14.09	11.59	14.18	11.31	6.38	2.69
	N	10	10	10	10	10	10
Na	Mean	149.60	150.10	148.50	147.20[c]	148.60	148.10
(mmol/L)	S.D.	1.65	2.38	1.78	2.30	2.07	1.73

	N	10	10	10	10	10	10
		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
K	Mean	9.43	10.12	10.36	11.27	8.95	10.39
(mmol/L)	S.D.	2.13	1.56	2.54	2.16	2.17	2.05
	N	10	10	10	10	10	10
Cl	Mean	104.70	104.60	104.00	103.70	103.60	104.20
(mmol/L)	S.D.	1.77	1.07	1.25	1.25	1.17	1.75
	N	10	10	10	10	10	10

a. Significantly different than the 415 mg/kg dose group, P = 0.03
b. Significantly different than the 100 and 210 mg/kg dose groups, P = 0.01
c. Significantly different than the 100 mg/kg dose groups, P = 0.03.

Table H-4
14-Day Individual Clinical Chemistry
Female Rats

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Notes
	14-694	3.3	117	60	110	16	11.7	74	0.6	3.3	244	14.0	6.6	34	150	9.2	104	Serum hemolyzed
Corn Oil	14-695	3.3	181	104	107	22	11.7	61	0.7	3.2	188	13.9	6.4	53	151	12.6	104	
Control	14-700	3.3	130	85	91	24	11.1	57	0.7	3.3	100	12.3	6.6	29	149	9.6	106	
	14-701	3.1	124	37	110	14	10.3	37	0.5	3.3	102	10.7	6.3	21	148	5.2	108	
	14-716	3.4	110	48	192	20	11.4	56	0.7	3.1	77	18.8	6.5	45	149	11.6	104	PHOS 1:1 dilution; Serum hemolyzed
	14-717	1.9	133	58	93	19	11.3	61	0.8	4.0	113	13.9	5.9	35	151	9.3	106	
	14-730	3.4	169	84	83	29	11.7	84	0.7	3.5	119	13.3	7.0	37	153	10.7	103	
	14-731	3.2	122	111	552	17	11.1	50	0.8	3.1	100	17.0	6.3	72	148	10.5	106	PHOS 1:1 dilution; Serum hemolyzed
	14-732	2.9	162	89	113	21	11.3	76	0.7	3.0	97	12.6	5.9	36	149	7.9	102	
	14-733	3.2	192	82	91	15	11.6	77	0.6	3.4	175	12.6	6.7	40	148	7.7	104	
	Mean	**3.10**	**144.00**	**75.80**	**154.20**	**19.70**	**11.32**	**63.30**	**0.68**	**3.32**	**131.50**	**13.91**	**6.42**	**40.20**	**149.60**	**9.43**	**104.70**	
	S.D.	**0.45**	**29.27**	**24.14**	**143.10**	**4.57**	**0.43**	**14.39**	**0.09**	**0.28**	**52.97**	**2.36**	**0.34**	**14.09**	**1.65**	**2.13**	**1.77**	
	14-692	3.5	141	64	102	22	11.3	59	0.9	3.2	104	18.2	6.7	52	151	10.9	106	PHOS 1:1 dilution
100 mg/kg	14-693	3.5	189	96	69	21	11.7	74	0.8	3.8	95	13.9	7.3	63	152	10.9	104	
	14-702	3.3	125	67	81	21	10.6	66	0.9	3.0	104	15.3	6.4	36	153	11.7	104	
	14-703	3.0	109	82	87	22	10.6	59	0.6	3.2	78	12.6	6.2	41	151	8.7	106	
	14-714	3.4	242	90	98	20	12.2	86	0.9	3.2	249	13.9	6.6	37	149	10.9	103	
	14-715	3.4	188	91	98	17	11.2	72	0.8	3.0	151	14.1	6.3	44	153	10.6	104	
	14-740	3.1	184	79	94	30	11.8	83	0.9	3.1	86	14.5	6.2	28	147	11.5	105	
	14-741	3.1	153	87	163	26	11.7	64	0.8	2.9	80	15.4	6.0	63	149	9.6	104	Serum hemolyzed
	14-742	3.3	162	89	98	25	11.6	93	0.8	3.7	91	14.1	6.9	50	150	9.9	104	
	14-743	2.9	93	69	126	21	10.9	66	0.5	2.9	81	10.4	5.8	39	146	6.5	106	
	Mean	3.5	141	64	102	22	11.3	59	0.9	3.2	104	18.2	6.7	52	151	10.9	106	PHOS 1:1 dilution
	S.D.	3.5	189	96	69	21	11.7	74	0.8	3.8	95	13.9	7.3	63	152	10.9	104	
	14-690	3.1	180	83	100	26	12.1	83	0.8	3.1	91	15.4	6.2	65	148	14.2	105	
210 mg/kg	14-691	3.1	160	81	98	15	11.2	61	0.6	3.2	68	16.6	6.2	38	152	13.4	104	PHOS 1:1 dilution
	14-718	2.9	149	62	104	19	10.9	48	0.6	3.3	89	13.7	6.2	33	151	7.9	104	
	14-719	2.9	204	71	93	25	10.7	55	0.7	3.2	128	11.6	6.1	25	148	7.7	106	
	14-744	3.4	155	55	90	21	12.0	86	0.7	3.0	123	13.3	6.4	34	148	11.2	105	
	14-745	3.6	197	139	134	28	12.5	83	0.8	3.4	83	15.6	7.0	62	146	ND	105	PHOS 1:1 dilution
	14-746	3.3	166	90	408	23	12.1	78	0.9	3.4	60	19.4	6.8	39	147	11.9	103	PHOS 1:1 dilution
	14-747	2.8	197	54	102	20	11.3	96	0.8	3.1	94	12.5	5.8	56	148	8.5	103	
	14-748	3.2	223	83	114	22	12.1	79	0.9	3.4	88	15.7	6.6	48	148	10.8	102	
	14-749	3.1	163	60	97	24	11.5	72	0.7	3.2	102	13.8	6.3	28	149	7.6	103	
	Mean	**3.14**	**179.40**	**77.80**	**134.00**	**22.30**	**11.64**	**74.10**	**0.75**	**3.23**	**92.60**	**14.76**	**6.36**	**42.80**	**148.50**	**10.36**	**104.00**	
	S.D.	**0.25**	**24.65**	**25.07**	**97.09**	**3.77**	**0.60**	**15.06**	**0.11**	**0.14**	**21.25**	**2.27**	**0.35**	**14.18**	**1.78**	**2.54**	**1.25**	
	14-708	3.1	141	73	95	18	11.1	80	0.7	3.2	92	14.3	6.3	31	151	10.4	104	
415 mg/kg	14-709	3.3	198	71	79	19	11.7	74	0.8	3.2	179	14.0	6.5	53	149	12.2	103	
	14-710	3.1	85	53	70	25	10.7	55	0.7	2.9	105	10.8	6.0	26	146	7.4	104	
	14-711	3.3	147	85	117	30	11.4	61	0.9	3.2	110	15.5	6.5	59	147	14.2	102	
	14-720	3.4	149	88	186	25	11.1	52	0.8	3.0	64	16.5	6.4	33	143	9.5	106	PHOS 1:1 dilution
	14-721	3.0	208	60	79	21	12.4	66	0.7	3.2	95	17.7	6.2	48	146	14.7	104	PHOS 1:1 dilution
	14-722	3.4	185	99	116	27	12.1	90	0.9	3.6	99	15.9	7.0	31	148	11.1	102	
	14-723	3.5	231	96	113	32	11.9	84	0.9	3.5	66	14.2	7.0	53	149	11.3	104	
	14-728	3.2	153	67	77	18	12.4	64	0.6	2.9	271	13.8	6.0	41	148	10.0	103	
	14-729	3.0	243	127	115	30	12.2	77	0.7	3.1	247	13.6	6.2	44	145	11.9	105	
	Mean	**3.23**	**174.00**	**81.90**	**104.70**	**24.50**	**11.70**	**70.30**	**0.77**	**3.18**	**132.80**	**14.63**	**6.41**	**41.90**	**147.20**	**11.27**	**103.70**	
	S.D.	**0.18**	**47.92**	**21.88**	**34.08**	**5.28**	**0.60**	**12.66**	**0.11**	**0.23**	**73.79**	**1.90**	**0.36**	**11.31**	**2.30**	**2.16**	**1.25**	

Toxicology Study No. S.0024883, July-September 2014

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CA (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (mg/dL)	GLU (mg/dL)	PHOS (mg/dL)	TP (g/dL)	TRIG (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Notes
	14-698	3.0	171	108	204	15	10.4	55	0.5	3.1	126	11.4	6.1	18	147	5.6	105	
830 mg/kg	14-699	3.5	146	101	81	28	11.3	75	0.8	3.2	73	18.0	6.7	31	151	11.2	104	PHOS 1:1 dilution
	14-704	3.1	98	76	79	19	10.6	44	0.5	3.3	87	10.1	6.4	22	147	6.6	103	
	14-705	2.9	131	84	135	18	10.4	64	0.6	3.2	110	11.7	6.1	22	145	5.6	103	
	14-712	3.3	174	86	78	17	11.4	74	0.7	3.4	121	14.2	6.7	27	149	9.4	102	
	14-713	3.1	156	78	93	22	11.5	67	0.7	3.3	166	13.3	6.4	27	148	9.5	102	
	14-726	3.0	155	63	100	18	11.1	81	0.7	3.1	82	14.1	6.1	34	150	10.0	105	
	14-727	3.3	142	80	105	21	11.4	68	0.8	3.2	83	16.1	6.5	36	152	10.6	105	PHOS 1:1 dilution
	14-736	3.3	213	94	120	23	11.8	64	0.8	3.4	105	15.6	6.7	37	149	10.1	104	
	14-737	3.0	190	92	98	18	11.8	108	0.7	3.3	199	15.4	6.3	30	148	10.9	103	
	Mean	**3.15**	**157.60**	**86.20**	**109.30**	**19.90**	**11.17**	**70.00**	**0.68**	**3.25**	**115.20**	**13.99**	**6.40**	**28.40**	**148.60**	**8.95**	**103.60**	
	S.D.	**0.18**	**40.84**	**17.19**	**17.38**	**2.95**	**0.37**	**17.93**	**0.14**	**0.20**	**39.22**	**2.01**	**0.33**	**29.07**	**1.78**	**1.84**	**1.40**	
	14-696	3.7	129	52	74	19	11.6	82	0.8	3.3	144	15.7	7.0	38	148	13.3	104	
1250 mg/kg	14-697	3.2	153	75	118	23	11.0	60	0.5	3.3	94	15.0	6.6	37	150	10.5	105	
	14-706	3.2	124	85	84	14	10.8	64	0.7	3.1	96	11.9	6.3	34	146	9.4	101	
	14-707	3.1	220	94	105	18	10.8	59	0.5	3.1	115	10.0	6.3	35	148	6.4	104	
	14-724	3.4	196	93	89	18	11.3	63	0.6	3.1	252	12.6	6.5	37	148	11.0	104	
	14-725	3.4	215	89	112	17	11.7	73	0.6	3.1	185	14.6	6.5	30	152	9.0	104	
	14-734	3.6	146	91	98	23	11.7	61	0.8	3.8	85	15.6	7.3	33	147	11.4	105	
	14-735	3.3	145	55	94	21	11.9	82	0.8	3.0	82	17.6	6.3	34	147	13.3	106	PHOS 1:1 dilution
	14-738	3.2	247	90	110	20	12.4	92	0.7	3.3	260	13.1	6.5	32	148	9.8	102	
	14-739	3.6	116	99	120	17	11.8	57	0.7	2.9	258	13.1	6.5	31	147	9.8	107	
	Mean	**3.37**	**169.10**	**82.30**	**100.40**	**19.00**	**11.50**	**69.30**	**0.67**	**3.20**	**157.10**	**13.92**	**6.58**	**34.10**	**148.10**	**10.39**	**104.20**	
	S.D.	**0.16**	**57.77**	**31.06**	**12.72**	**3.65**	**0.47**	**9.52**	**0.14**	**0.11**	**33.93**	**2.13**	**0.24**	**15.36**	**2.50**	**1.42**	**1.29**	

APPENDIX I
SUMMARY OF 14-DAY HEMATOLOGY AND INDIVIDUAL DATA

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table I-1
14-Day Hematology Summary
Male Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
WBC	Mean	11.38	11.91	10.64	12.18	11.84	11.45
(K/uL)	S.D.	2.14	2.84	2.83	2.93	2.73	2.86
	N	8	10	10	10	10	10
NEU	Mean	1.88	1.94	1.81	1.93	1.90	1.92
(K/uL)	S.D.	0.55	0.69	0.53	0.60	0.65	0.50
	N	8	10	10	10	10	10
NEU	Mean	16.51	16.36	17.29	15.85	15.94	17.34
(%N)	S.D.	3.60	4.15	3.62	3.58	4.08	4.84
	N	8	10	10	10	10	10
LYM	Mean	9.12	9.47	8.21	9.73	9.13	8.93
(K/uL)	S.D.	1.74	2.40	2.39	2.59	2.00	2.70
	N	8	10	10	10	10	10
LYM	Mean	79.96	79.44	77.02	79.56	77.60	77.11
(%L)	S.D.	4.02	6.55	5.99	5.64	7.98	7.03
	N	8	10	10	10	10	10
MONO	Mean	0.09	0.13	0.24	0.16	0.39	0.22
(K/uL)	S.D.	0.08	0.17	0.31	0.21	0.55	0.27
	N	8	10	10	10	10	10
MONO	Mean	0.78	1.04	2.09	1.59	2.90	2.02
(%M)	S.D.	0.60	1.21	2.54	2.34	3.81	2.67
	N	8	10	10	10	10	10
EOS	Mean	0.10	0.10	0.08	0.08	0.09	0.09
(K/uL)	S.D.	0.02	0.05	0.04	0.05	0.04	0.06
	N	8	10	10	10	10	10
EOS	Mean	0.96	0.84	0.72	0.67	0.74	0.80
(%E)	S.D.	0.31	0.34	0.37	0.28	0.27	0.45
	N	8	10	10	10	10	10
BASO	Mean	0.20	0.28	0.31	0.27	0.35	0.31
(K/uL)	S.D.	0.10	0.37	0.18	0.16	0.30	0.19
	N	8	10	10	10	10	10
BASO	Mean	1.77	2.30	2.90	2.34	2.83	2.72
(%B)	S.D.	0.71	2.54	1.44	1.56	1.98	1.71
	N	8	10	10	10	10	10
RBC	Mean	7.84	7.88	8.01	8.08	8.16	8.11
(M/uL)	S.D.	0.28	0.32	0.48	0.38	0.72	0.52
	N	8	10	10	10	10	10

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
HGB	Mean	16.48	16.31	16.67	16.98	17.34	16.30
(g/dL)	S.D.	0.52	0.58	0.98	0.93	1.24	1.22
	N	8	10	10	10	10	10
HCT	Mean	46.95	47.02	47.33	48.45	49.32	46.96
(%)	S.D.	1.47	1.90	2.50	2.35	3.25	3.51
	N	8	10	10	10	10	10
MCV	Mean	59.93	59.71	59.14	60.02	60.58	57.87
(fL)	S.D.	2.36	1.73	1.31	1.57	2.90	1.33
	N	8	10	10	10	10	10
MCH	Mean	21.00	20.76	20.81	21.05	21.31[a]	20.11
(pg)	S.D.	0.71	0.60	0.50	0.63	0.95	0.51
	N	8	10	10	10	10	10
MCHC	Mean	35.08	34.77	35.18	35.06	35.16	34.73
(g/dL)	S.D.	0.55	0.62	0.55	0.58	0.38	0.40
	N	8	10	10	10	10	10
RDW	Mean	15.85	16.19	16.55	16.57	16.56	17.10
(%)	S.D.	1.01	0.83	0.67	0.83	1.17	0.99
	N	8	10	10	10	10	10
PLT	Mean	1136.13	1096.10	1123.80	1114.30	1036.00	1075.30
(K/uL)	S.D.	201.06	196.12	144.24	93.51	226.37	174.61
	N	8	10	10	10	10	10
MPV	Mean	5.16	5.08	5.21	4.98	4.89	4.89
(fL)	S.D.	0.40	0.57	0.83	0.31	0.28	0.53
	N	8	10	10	10	10	10

a. Significantly different from 415 mg/kg dose group, P = 0.0006

Table I-2
14-Day Individual Hematology (White Cell Data)
Male Rats

Dose	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	Notes
	14-635	10.90	1.500	13.800	8.870	81.400	0.178	1.640	0.116	1.070	0.234	2.140	
Corn Oil	14-639	13.60	2.720	20.000	10.700	78.700	0.032	0.239	0.059	0.433	0.085	0.627	
Control	14-640	10.40	1.440	13.800	8.720	83.500	0.020	0.188	0.113	1.080	0.147	1.410	
	14-657	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Sample Clotted
	14-658	11.10	1.580	14.200	9.250	83.200	0.061	0.548	0.089	0.799	0.131	1.180	
	14-673	7.67	1.580	20.600	5.800	75.500	0.036	0.463	0.118	1.540	0.144	1.880	
	14-674	10.80	1.360	12.500	9.150	84.500	0.041	0.381	0.100	0.924	0.180	1.660	
	14-687	11.80	2.540	21.500	8.680	73.200	0.183	1.540	0.105	0.886	0.339	2.860	
	14-688	14.80	2.320	15.700	11.800	79.700	0.183	1.240	0.139	0.944	0.355	2.410	
	Mean	**11.38**	**1.88**	**16.51**	**9.12**	**79.96**	**0.09**	**0.78**	**0.10**	**0.96**	**0.20**	**1.77**	
	S.D.	**2.14**	**0.551**	**3.597**	**1.738**	**4.017**	**0.075**	**0.596**	**0.024**	**0.310**	**0.099**	**0.711**	
	14-641	14.30	3.350	23.400	8.940	62.500	0.573	4.010	0.139	0.975	1.290	9.030	
100 mg/kg	14-642	10.20	1.720	16.800	8.370	82.000	0.023	0.228	0.063	0.615	0.035	0.342	
	14-645	11.20	2.440	21.700	8.570	76.400	0.018	0.158	0.074	0.656	0.120	1.070	
	14-646	11.70	2.210	18.900	9.180	78.600	0.023	0.200	0.178	1.520	0.086	0.737	
	14-653	13.60	2.170	16.000	11.200	82.100	0.053	0.391	0.122	0.894	0.077	0.567	
	14-654	5.13	0.828	16.100	4.030	78.500	0.098	1.900	0.029	0.569	0.149	2.910	
	14-663	12.70	1.800	14.200	10.700	83.800	0.024	0.187	0.039	0.304	0.195	1.530	
	14-664	12.30	1.200	9.740	10.400	84.600	0.171	1.400	0.141	1.150	0.379	3.090	
	14-677	15.60	1.860	12.000	13.200	84.500	0.194	1.250	0.115	0.737	0.242	1.560	
	14-678	12.40	1.820	14.800	10.100	81.400	0.080	0.647	0.119	0.965	0.271	2.200	
	Mean	**11.91**	**1.940**	**16.364**	**9.469**	**79.440**	**0.126**	**1.037**	**0.102**	**0.839**	**0.284**	**2.304**	
	S.D.	**2.84**	**0.686**	**4.148**	**2.396**	**6.553**	**0.169**	**1.210**	**0.048**	**0.342**	**0.368**	**2.544**	
	14-629	9.19	1.650	17.900	7.320	79.600	0.037	0.405	0.024	0.266	0.163	1.770	
210 mg/kg	14-630	8.16	1.570	19.300	6.330	77.700	0.026	0.323	0.057	0.702	0.164	2.010	
	14-649	11.10	2.680	24.100	7.470	67.200	0.482	4.340	0.052	0.471	0.436	3.920	
	14-650	9.58	1.160	12.200	8.190	85.600	0.022	0.229	0.071	0.746	0.124	1.300	
	14-665	15.30	1.870	12.200	13.000	85.000	0.069	0.448	0.058	0.376	0.309	2.010	
	14-666	10.10	1.770	17.500	6.960	68.900	0.646	6.390	0.147	1.450	0.587	5.800	
	14-675	5.41	0.939	17.400	4.250	78.600	0.055	1.010	0.023	0.420	0.139	2.560	
	14-676	11.30	2.220	19.700	8.570	76.000	0.059	0.519	0.122	1.080	0.301	2.670	
	14-683	12.80	2.340	18.200	10.000	78.000	0.117	0.910	0.085	0.660	0.286	2.230	
	14-684	13.50	1.950	14.400	9.960	73.600	0.850	6.280	0.135	0.995	0.633	4.680	
	Mean	**10.64**	**1.815**	**17.290**	**8.205**	**77.020**	**0.236**	**2.085**	**0.077**	**0.717**	**0.314**	**2.895**	
	S.D.	**2.83**	**0.526**	**3.616**	**2.391**	**5.991**	**0.306**	**2.544**	**0.044**	**0.368**	**0.184**	**1.440**	
	14-625	13.00	2.410	18.500	9.410	72.500	0.517	3.980	0.047	0.365	0.603	4.640	
415 mg/kg	14-626	16.60	2.090	12.600	14.100	84.800	0.043	0.260	0.104	0.626	0.286	1.720	
	14-633	16.10	2.530	15.800	12.800	80.000	0.140	0.872	0.187	1.160	0.355	2.210	
	14-634	10.50	2.190	20.800	7.880	74.700	0.095	0.904	0.095	0.904	0.285	2.700	
	14-643	11.20	2.040	18.300	8.900	79.700	0.011	0.095	0.085	0.762	0.134	1.200	
	14-644	10.70	0.901	8.420	9.360	87.500	0.170	1.590	0.036	0.337	0.232	2.160	
	14-667	14.80	2.660	18.000	11.900	80.300	0.026	0.174	0.094	0.635	0.135	0.909	
	14-668	9.33	1.240	13.300	7.900	84.700	0.052	0.555	0.026	0.284	0.110	1.180	
	14-681	11.90	1.950	16.400	9.670	81.400	0.016	0.134	0.102	0.863	0.139	1.170	
	14-682	7.62	1.250	16.400	5.330	70.000	0.562	7.370	0.058	0.757	0.420	5.510	
	Mean	**12.18**	**1.926**	**15.852**	**9.725**	**79.560**	**0.163**	**1.593**	**0.083**	**0.669**	**0.270**	**2.340**	
	S.D.	**2.93**	**0.599**	**3.575**	**2.588**	**5.636**	**0.205**	**2.341**	**0.046**	**0.280**	**0.157**	**1.562**	

Toxicology Study No. S.0024883, July-September 2014

Dose	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	Notes
	14-655	11.30	1.910	16.800	9.170	80.900	0.031	0.271	0.040	0.350	0.189	1.670	
830 mg/kg	14-656	10.50	2.130	20.200	6.780	64.400	0.893	8.480	0.141	1.340	0.593	5.630	
	14-661	11.40	1.670	14.600	9.150	80.400	0.179	1.580	0.062	0.546	0.324	2.840	
	14-662	10.00	1.880	18.800	7.790	77.700	0.087	0.865	0.091	0.911	0.175	1.740	
	14-669	7.69	0.869	11.300	6.550	85.100	0.025	0.327	0.051	0.668	0.200	2.600	
	14-670	11.90	1.680	14.100	9.960	83.800	0.031	0.261	0.092	0.773	0.126	1.060	
	14-679	13.00	2.800	21.600	8.330	64.200	1.040	8.000	0.090	0.696	0.705	5.440	
	14-680	17.40	1.970	11.400	12.800	73.600	1.510	8.670	0.155	0.892	0.957	5.510	
	14-685	14.90	2.960	19.900	11.800	78.900	0.025	0.168	0.075	0.505	0.085	0.570	
	14-686	10.30	1.100	10.700	8.930	87.000	0.039	0.379	0.074	0.724	0.127	1.240	
	Mean	**11.84**	**1.897**	**15.940**	**9.126**	**77.600**	**0.386**	**2.900**	**0.087**	**0.741**	**0.348**	**2.830**	
	S.D.	**2.73**	**0.649**	**4.077**	**1.998**	**7.981**	**0.549**	**3.810**	**0.037**	**0.272**	**0.299**	**1.977**	
	14-627	14.60	1.670	11.400	12.500	85.500	0.132	0.904	0.059	0.405	0.258	1.770	
1250 mg/kg	14-628	12.50	2.710	21.800	9.450	75.900	0.046	0.366	0.072	0.576	0.174	1.400	
	14-631	13.70	1.890	13.800	11.300	82.300	0.017	0.127	0.163	1.190	0.358	2.610	
	14-632	11.20	2.540	22.600	8.290	73.800	0.128	1.140	0.046	0.408	0.222	1.980	
	14-637	14.10	2.220	15.700	11.600	81.700	0.026	0.182	0.156	1.100	0.180	1.270	
	14-638	6.02	0.990	16.500	4.880	81.200	0.021	0.356	0.018	0.300	0.103	1.710	
	14-651	8.93	1.930	21.600	6.770	75.800	0.041	0.464	0.055	0.618	0.135	1.510	
	14-652	12.80	1.550	12.200	10.300	80.500	0.416	3.260	0.065	0.509	0.451	3.530	
	14-671	8.06	1.980	24.500	4.890	60.600	0.617	7.650	0.121	1.500	0.461	5.710	
	14-672	12.60	1.670	13.300	9.280	73.800	0.727	5.790	0.175	1.390	0.718	5.710	
	Mean	**11.45**	**1.915**	**17.340**	**8.926**	**77.110**	**0.217**	**2.024**	**0.093**	**0.800**	**0.306**	**2.720**	
	S.D.	**2.86**	**0.498**	**4.841**	**2.699**	**7.026**	**0.269**	**2.674**	**0.056**	**0.448**	**0.192**	**1.710**	

Table I-3
14-Day Individual Hematology (Red Cell Data)
Male Rats

Dose	Animal ID	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	Notes
	14-635	7.88	16.50	47.5	60.3	20.9	34.7	15.1	1009.0	5.29	
Corn Oil	14-639	7.50	16.20	47.4	63.2	21.5	34.1	16.4	1185.0	5.04	
Control	14-640	7.50	16.70	47.6	63.5	22.3	35.1	15.4	1117.0	5.39	
	14-657	ND	ND	ND	ND	ND	ND	ND	ND	ND	Sample Clotted
	14-658	8.01	16.40	46.4	57.9	20.4	35.3	14.7	1006.0	5.08	
	14-673	7.86	15.70	44.7	56.8	20.0	35.1	18.0	820.0	4.96	
	14-674	7.64	16.00	45.2	59.2	20.9	35.4	15.6	1163.0	5.10	
	14-687	8.02	17.10	47.5	59.3	21.3	36.0	15.9	1475.0	5.91	
	14-688	8.32	17.20	49.3	59.2	20.7	34.9	15.7	1314.0	4.51	
	Mean	**7.84**	**16.48**	**46.95**	**59.93**	**21.00**	**35.08**	**15.85**	**1136.13**	**5.16**	
	S.D.	**0.28**	**0.52**	**1.5**	**2.4**	**0.7**	**0.6**	**1.0**	**201.1**	**0.40**	
	14-641	7.31	15.20	43.8	59.9	20.8	34.8	17.3	751.0	5.82	
100 mg/kg	14-642	8.09	16.90	50.0	61.8	20.9	33.8	16.5	873.0	5.00	
	14-645	7.88	15.60	45.5	57.7	19.9	34.4	15.5	1178.0	4.44	
	14-646	7.87	16.90	47.4	60.2	21.5	35.8	16.8	1342.0	5.09	
	14-653	7.69	15.90	46.0	59.8	20.7	34.6	15.4	1202.0	6.27	
	14-654	7.85	16.30	46.2	58.8	20.8	35.4	15.2	857.0	5.13	
	14-663	7.47	16.30	46.5	62.3	21.8	35.1	17.0	1228.0	4.72	
	14-664	8.33	16.50	47.5	57.0	19.9	34.9	15.0	1137.0	4.53	
	14-677	8.17	16.90	49.9	61.1	20.8	33.9	16.4	1161.0	4.92	
	14-678	8.10	16.60	47.4	58.5	20.5	35.0	16.8	1232.0	4.88	
	Mean	**7.88**	**16.31**	**47.0**	**59.7**	**20.8**	**34.8**	**16.2**	**1096.1**	**5.08**	
	S.D.	**0.32**	**0.58**	**1.9**	**1.7**	**0.6**	**0.6**	**0.8**	**196.1**	**0.57**	
	14-629	7.98	16.30	47.4	59.4	20.4	34.4	17.0	989.0	5.00	
210 mg/kg	14-630	7.40	15.10	42.8	57.8	20.4	35.2	14.9	930.0	4.75	
	14-649	7.87	16.50	46.5	59.1	21.0	35.4	16.5	1061.0	4.99	
	14-650	8.43	17.20	48.9	58.0	20.4	35.1	16.2	1235.0	5.02	
	14-665	7.64	16.30	46.7	61.2	21.3	34.8	17.1	1082.0	4.61	
	14-666	8.89	17.90	50.4	56.7	20.2	35.6	17.0	1435.0	7.43	
	14-675	7.62	15.60	45.2	59.4	20.4	34.4	17.2	1103.0	4.92	
	14-676	7.57	16.20	45.4	60.0	21.4	35.7	16.6	1087.0	5.73	
	14-683	8.41	18.10	50.2	59.7	21.5	36.1	16.6	1237.0	4.89	
	14-684	8.28	17.50	49.8	60.1	21.1	35.1	16.4	1079.0	4.78	
	Mean	**8.01**	**16.67**	**47.3**	**59.1**	**20.8**	**35.2**	**16.6**	**1123.8**	**5.21**	
	S.D.	**0.48**	**0.98**	**2.5**	**1.3**	**0.5**	**0.5**	**0.7**	**144.2**	**0.83**	
	14-625	7.87	16.40	47.5	60.4	20.9	34.6	17.0	1141.0	5.38	
415 mg/kg	14-626	8.07	16.90	49.1	60.9	21.0	34.5	17.4	1122.0	4.76	
	14-633	7.91	16.50	48.3	61.0	20.9	34.2	16.5	1114.0	5.12	
	14-634	7.72	17.10	48.3	62.6	22.1	35.4	17.0	1207.0	4.92	
	14-643	8.47	16.70	48.0	56.7	19.8	34.9	17.4	1147.0	4.80	
	14-644	8.18	17.20	49.5	60.5	21.0	34.7	14.6	1027.0	4.73	
	14-667	7.90	16.30	46.5	58.9	20.6	35.1	16.0	1011.0	5.08	
	14-668	8.98	19.50	54.4	60.5	21.8	35.9	16.4	1140.0	5.50	
	14-681	7.81	16.70	46.4	59.4	21.4	35.9	17.0	1274.0	5.02	
	14-682	7.86	16.50	46.5	59.3	21.0	35.4	16.4	960.0	4.50	
	Mean	**8.08**	**16.98**	**48.5**	**60.0**	**21.1**	**35.1**	**16.6**	**1114.3**	**4.98**	
	S.D.	**0.38**	**0.93**	**2.4**	**1.6**	**0.6**	**0.6**	**0.8**	**93.5**	**0.31**	

Dose	Animal ID	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	Notes
	14-655	7.84	17.20	48.2	61.5	22.0	35.8	18.4	1253.0	4.84	
830 mg/kg	14-656	7.85	17.90	51.3	65.3	22.8	34.9	14.2	1110.0	5.29	
	14-661	8.43	17.60	49.9	59.2	20.9	35.3	17.8	1176.0	4.95	
	14-662	9.81	19.90	56.0	57.1	20.2	35.4	16.7	430.0	5.14	
	14-669	8.47	17.20	48.5	57.3	20.4	35.5	16.8	1156.0	4.63	
	14-670	7.42	15.90	45.3	61.1	21.4	35.0	16.8	1076.0	4.76	
	14-679	7.53	15.50	44.9	59.6	20.6	34.6	15.5	1081.0	4.81	
	14-680	7.63	16.40	47.4	62.1	21.6	34.7	16.8	1074.0	5.25	
	14-685	8.73	17.90	50.5	57.9	20.5	35.4	15.9	1001.0	4.83	
	14-686	7.91	17.90	51.2	64.7	22.7	35.0	16.7	1003.0	4.38	
	Mean	**8.16**	**17.34**	**49.3**	**60.6**	**21.3**	**35.2**	**16.6**	**1036.0**	**4.89**	
	S.D.	**0.72**	**1.24**	**3.3**	**2.9**	**0.9**	**0.4**	**1.2**	**226.4**	**0.28**	
	14-627	8.43	17.70	50.8	60.3	21.0	34.9	17.9	980.0	5.80	
1250 mg/kg	14-628	8.28	17.00	48.6	58.7	20.5	35.0	16.8	1259.0	4.59	
	14-631	7.82	15.50	45.2	57.8	19.9	34.4	15.6	853.0	4.32	
	14-632	9.16	18.10	52.9	57.7	19.8	34.3	17.6	1065.0	4.84	
	14-637	7.44	14.80	43.1	57.9	19.9	34.3	16.8	1057.0	4.33	
	14-638	7.99	15.50	44.4	55.6	19.4	34.8	17.6	1166.0	4.41	
	14-651	8.68	17.70	50.7	58.4	20.4	34.9	15.9	1118.0	5.03	
	14-652	7.77	15.10	44.2	56.8	19.5	34.3	18.0	949.0	4.94	
	14-671	7.82	16.10	46.1	58.9	20.6	34.9	16.2	885.0	5.73	
	14-672	7.71	15.50	43.6	56.6	20.1	35.5	18.6	1421.0	4.88	
	Mean	**8.11**	**16.30**	**47.0**	**57.9**	**20.1**	**34.7**	**17.1**	**1075.3**	**4.89**	
	S.D.	**0.52**	**1.22**	**3.5**	**1.3**	**0.5**	**0.4**	**1.0**	**174.6**	**0.53**	

Table I-4
14-Day Hematology Summary
Female Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
WBC	Mean	4.75	7.72	7.66	7.49	7.53	9.06
(K/uL)	S.D.	1.77	2.69	3.73	2.69	2.83	3.90
	N	10	10	10	10	10	10
NEU	Mean	0.80	1.04	1.08	0.87	0.97	0.98
(K/uL)	S.D.	0.35	0.58	0.48	0.36	0.56	0.45
	N	10	10	10	10	10	10
NEU	Mean	17.41	13.37	15.43	11.54	12.45	12.12
(%N)	S.D.	4.77	4.29	5.23	2.98	3.45	7.50
	N	10	10	10	10	10	10
LYM	Mean	3.65	6.36	6.25	6.42	6.29	7.40
(K/uL)	S.D.	1.43	2.18	3.24	2.20	2.25	4.31
	N	10	10	10	10	10	10
LYM	Mean	76.21	82.55	79.51	84.43[a]	83.98[a]	84.03[a]
(%L)	S.D.	4.43	3.56	6.88	2.88	3.66	7.02
	N	10	10	10	10	10	10
MONO	Mean	0.11	0.06	0.07	0.06	0.04	0.06
(K/uL)	S.D.	0.14	0.06	0.04	0.04	0.03	0.04
	N	10	10	10	10	10	10
MONO	Mean	2.20	0.78	1.53	0.83	0.50	0.70
(%M)	S.D.	2.34	0.57	1.80	0.49	0.30	0.48
	N	10	10	10	10	10	10
EOS	Mean	0.06	0.08	0.10	0.07	0.06	0.08
(K/uL)	S.D.	0.03	0.05	0.07	0.03	0.03	0.05
	N	10	10	10	10	10	10
EOS	Mean	1.10	1.08	1.13	0.93	0.84	0.90
(%E)	S.D.	0.37	0.68	0.41	0.26	0.27	0.44
	N	10	10	10	10	10	10
BASO	Mean	0.14	0.18	0.17	0.16	0.16	0.21
(K/uL)	S.D.	0.11	0.13	0.11	0.06	0.07	0.15
	N	10	10	10	10	10	10
BASO	Mean	3.06	2.23	2.40	2.11	2.25	2.24
(%B)	S.D.	1.82	1.26	1.04	1.00	0.81	1.02
	N	10	10	10	10	10	10
RBC	Mean	8.12	8.31	8.31	7.84	8.22	8.15
(M/uL)	S.D.	0.46	0.62	0.47	0.44	0.62	0.31
	N	10	10	10	10	10	10
HGB	Mean	16.53	17.18	17.11	16.48	16.74	16.51
(g/dL)	S.D.	0.89	0.84	0.83	1.16	1.27	0.78
	N	10	10	10	10	10	10
HCT	Mean	46.81	48.63	48.43	46.46	47.48	46.69
(%)	S.D.	2.58	2.66	2.29	3.31	3.51	1.88
	N	10	10	10	10	10	10

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MCV (fL)	Mean	57.67	58.58	58.29	59.24	57.85	57.33
	S.D.	1.04	1.74	1.38	2.22	2.74	1.34
	N	10	10	10	10	10	10
		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
MCH (pg)	Mean	20.35	20.70	20.59	21.02	20.39	20.28
	S.D.	0.33	0.67	0.72	0.83	0.97	0.58
	N	10	10	10	10	10	10
MCHC (g/dL)	Mean	35.32	35.34	35.34	35.52	35.26	35.33
	S.D.	0.37	0.67	0.63	0.49	0.49	0.45
	N	10	10	10	10	10	10
RDW (%)	Mean	15.32	15.53	15.63	15.36	15.52	15.86
	S.D.	0.40	0.96	0.83	0.49	0.91	1.35
	N	10	10	10	10	10	10
PLT (K/uL)	Mean	1078.80	1175.10	1005.70	1136.30	1156.40	1150.70
	S.D.	232.58	124.45	214.20	127.90	182.45	190.64
	N	10	10	10	10	10	10
MPV (fL)	Mean	5.23	5.21	5.11	5.04	4.90	5.18
	S.D.	0.48	0.49	0.39	0.28	0.32	0.48
	N	10	10	10	10	10	10

[a] Significantly different from control, P = 0.002

Table I-5
14-Day Individual Hematology (White Cell Data)
Female Rats

Dose	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	Notes
	14-694	6.72	0.95	14.10	4.88	72.60	0.43	6.40	0.09	1.41	0.37	5.50	
Corn Oil	14-695	4.60	0.50	11.00	3.47	75.50	0.27	5.78	0.05	1.07	0.31	6.71	
Control	14-700	2.20	0.48	21.90	1.60	73.00	0.03	1.15	0.01	0.30	0.08	3.61	
	14-701	2.62	0.56	21.40	1.86	70.90	0.11	4.23	0.02	0.89	0.07	2.54	
	14-716	3.06	0.61	19.90	2.31	75.50	0.03	1.06	0.02	0.77	0.08	2.74	
	14-717	4.06	0.68	16.70	3.21	78.90	0.05	1.10	0.06	1.52	0.07	1.74	
	14-730	7.06	0.95	13.40	5.86	83.00	0.04	0.52	0.08	1.12	0.14	1.96	
	14-731	6.31	1.66	26.30	4.48	71.00	0.05	0.71	0.09	1.37	0.04	0.56	
	14-732	6.24	0.94	15.00	5.03	80.50	0.05	0.81	0.08	1.34	0.15	2.34	
	14-733	4.63	0.67	14.40	3.76	81.20	0.01	0.20	0.06	1.24	0.14	2.94	
	Mean	**4.75**	**0.80**	**17.41**	**3.65**	**76.21**	**0.11**	**2.20**	**0.06**	**1.10**	**0.14**	**3.06**	
	S.D.	**2.69**	**0.58**	**4.29**	**2.18**	**3.56**	**0.06**	**0.57**	**0.05**	**0.68**	**0.13**	**1.26**	
	14-692	6.07	0.67	11.00	5.18	85.30	0.03	0.43	0.05	0.82	0.15	2.48	
100 mg/kg	14-693	3.64	0.67	18.40	2.87	78.80	0.02	0.50	0.08	2.27	0.00	0.06	
	14-702	6.14	0.73	11.80	5.08	82.80	0.10	1.68	0.02	0.26	0.21	3.46	
	14-703	4.08	0.50	12.30	3.41	83.50	0.03	0.83	0.05	1.10	0.09	2.31	
	14-714	10.30	2.02	19.60	8.10	78.60	0.03	0.29	0.05	0.52	0.10	0.95	
	14-715	7.22	0.77	10.70	6.20	85.90	0.02	0.32	0.06	0.88	0.16	2.25	
	14-740	10.80	2.16	20.00	8.23	76.20	0.18	1.64	0.13	1.18	0.11	0.99	
	14-741	8.30	1.00	12.10	6.87	82.80	0.05	0.58	0.19	2.27	0.19	2.29	
	14-742	10.50	0.95	9.05	8.92	84.60	0.15	1.37	0.10	0.92	0.43	4.05	
	14-743	10.10	0.88	8.78	8.74	87.00	0.02	0.21	0.06	0.57	0.35	3.46	
	Mean	**7.72**	**1.04**	**13.37**	**6.36**	**82.55**	**0.06**	**0.78**	**0.08**	**1.08**	**0.18**	**2.23**	
	S.D.	**1.77**	**0.35**	**4.77**	**1.43**	**4.43**	**0.14**	**2.34**	**0.03**	**0.37**	**0.11**	**1.82**	
	14-690	8.64	0.94	10.90	7.21	83.40	0.13	1.52	0.08	0.98	0.28	3.23	
210 mg/kg	14-691	2.69	0.41	15.20	2.09	77.90	0.09	3.48	0.02	0.90	0.07	2.45	
	14-718	2.12	0.58	27.30	1.31	61.70	0.13	5.92	0.02	1.05	0.09	4.04	
	14-719	11.50	1.40	12.20	9.41	82.20	0.10	0.89	0.17	1.48	0.37	3.21	
	14-744	9.99	1.95	19.50	7.74	77.50	0.08	0.75	0.07	0.74	0.15	1.48	
	14-745	9.21	1.08	11.80	7.61	82.60	0.08	0.83	0.15	1.57	0.30	3.21	
	14-746	9.38	1.38	14.70	7.69	82.00	0.03	0.30	0.10	1.11	0.18	1.87	
	14-747	12.70	1.23	9.68	11.10	87.20	0.03	0.23	0.24	1.89	0.13	1.01	
	14-748	6.87	1.28	18.60	5.42	78.90	0.04	0.55	0.07	1.03	0.07	0.98	
	14-749	3.52	0.51	14.40	2.87	81.70	0.03	0.82	0.02	0.53	0.09	2.55	
	Mean	**7.66**	**1.08**	**15.43**	**6.25**	**79.51**	**0.07**	**1.53**	**0.10**	**1.13**	**0.17**	**2.40**	
	S.D.	**3.73**	**0.48**	**5.23**	**3.24**	**6.88**	**0.04**	**1.80**	**0.07**	**0.41**	**0.11**	**1.04**	
	14-708	ND	1.01	12.00	7.06	84.00	0.08	0.95	0.06	0.75	0.20	2.37	
415 mg/kg	14-709	7.78	1.20	15.40	6.32	81.20	0.06	0.73	0.10	1.25	0.11	1.41	
	14-710	7.59	0.64	8.36	6.71	88.50	0.03	0.36	0.06	0.80	0.15	0.20	
	14-711	5.17	0.84	16.20	4.16	80.40	0.03	0.60	0.03	0.56	0.12	2.22	
	14-720	9.00	0.96	10.60	7.69	85.40	0.04	0.42	0.09	1.04	0.23	2.55	
	14-721	1.95	0.22	11.50	1.63	83.20	0.02	1.19	0.02	0.80	0.07	3.35	
	14-722	9.06	0.97	10.70	7.81	86.20	0.03	0.33	0.06	0.66	0.19	2.11	
	14-723	7.38	0.45	6.04	6.55	88.80	0.09	1.20	0.10	1.38	0.19	2.62	
	14-728	7.85	0.94	12.00	6.43	81.90	0.15	1.88	0.08	0.96	0.26	3.31	
	14-729	11.60	1.46	12.60	9.80	84.70	0.07	0.61	0.13	1.10	0.11	0.95	
	Mean	**7.49**	**0.87**	**11.54**	**6.42**	**84.43**	**0.06**	**0.83**	**0.07**	**0.93**	**0.16**	**2.11**	
	S.D.	**2.69**	**0.36**	**2.98**	**2.20**	**2.88**	**0.04**	**0.49**	**0.03**	**0.26**	**0.06**	**1.00**	

Toxicology Study No. S.0024883, July-September 2014

Dose	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	Notes
	14-698	7.06	0.83	11.70	5.95	84.40	0.02	0.28	0.07	0.95	0.19	2.68	
830 mg/kg	14-699	5.45	0.45	8.16	4.82	88.40	0.02	0.35	0.05	0.97	0.12	2.10	
	14-704	6.22	0.67	10.70	5.26	84.60	0.06	1.01	0.08	1.23	0.16	2.52	
	14-705	11.10	2.12	19.10	8.51	76.40	0.11	0.98	0.09	0.82	0.30	2.72	
	14-712	5.65	0.81	14.40	4.73	83.80	0.02	0.30	0.04	0.76	0.05	0.81	
	14-713	10.40	1.22	11.70	8.88	85.40	0.05	0.47	0.13	1.25	0.12	1.16	
	14-726	5.04	0.63	12.50	4.24	84.20	0.03	0.57	0.02	0.48	0.11	2.26	
	14-727	6.53	0.47	7.21	5.82	89.20	0.02	0.26	0.04	0.56	0.18	2.80	
	14-736	12.80	1.78	14.00	10.60	83.00	0.08	0.60	0.07	0.51	0.24	1.86	
	14-737	5.05	0.76	15.00	4.06	80.40	0.01	0.16	0.04	0.87	0.18	3.56	
	Mean	**7.53**	**0.97**	**12.45**	**6.29**	**83.98**	**0.04**	**0.50**	**0.06**	**0.84**	**0.16**	**2.25**	
	S.D.	**2.83**	**0.56**	**3.45**	**2.25**	**3.66**	**0.03**	**0.30**	**0.03**	**0.27**	**0.07**	**0.81**	
	14-696	5.89	1.60	27.20	4.17	70.80	0.03	0.46	0.04	0.59	0.06	0.94	
1250 mg/kg	14-697	7.40	1.70	22.90	5.55	75.00	0.04	0.48	0.04	0.51	0.08	1.12	
	14-706	8.38	1.07	12.70	6.77	80.80	0.16	1.91	0.16	1.88	0.22	2.66	
	14-707	5.54	0.48	8.58	4.82	86.90	0.04	0.68	0.05	0.82	0.17	3.00	
	14-724	13.00	0.84	6.47	11.80	90.20	0.08	0.65	0.16	1.21	0.20	1.50	
	14-725	4.45	0.31	6.98	0.39	88.20	0.03	0.56	0.05	1.04	0.15	3.26	
	14-734	15.80	0.89	5.63	14.10	89.50	0.07	0.42	0.11	0.71	0.60	3.78	
	14-735	7.14	0.74	10.30	6.20	86.90	0.03	0.45	0.07	0.93	0.10	1.42	
	14-738	14.10	0.83	5.85	13.00	91.80	0.05	0.33	0.04	0.31	0.24	1.67	
	14-739	8.93	1.31	14.60	7.16	80.20	0.10	1.10	0.09	0.98	0.27	3.07	
	Mean	**9.06**	**0.98**	**12.12**	**7.40**	**84.03**	**0.06**	**0.70**	**0.08**	**0.90**	**0.21**	**2.24**	
	S.D.	**3.90**	**0.45**	**7.50**	**4.31**	**7.02**	**0.04**	**0.48**	**0.05**	**0.44**	**0.15**	**1.02**	

Table I-6
14-Day Individual Hematology Data (Red Cell Data)
Female Rats

Dose	Animal ID	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	Notes
	14-694	7.78	16.00	45.30	58.30	20.50	35.20	15.20	1127.00	5.24	
Corn Oil	14-695	8.57	17.20	49.00	57.20	20.00	35.10	15.60	1091.00	5.33	
Control	14-700	7.78	15.60	44.20	56.80	20.00	35.30	15.40	1197.00	4.56	
	14-701	7.44	15.30	43.10	57.90	20.50	35.40	14.80	856.00	5.44	
	14-716	8.44	16.90	48.40	57.40	20.10	35.00	15.30	1087.00	5.01	
	14-717	7.93	15.80	44.90	56.70	20.00	35.30	15.70	1148.00	5.24	
	14-730	8.95	18.20	51.20	57.40	20.40	35.60	15.20	1327.00	5.19	
	14-731	7.86	16.50	47.30	60.20	21.00	34.90	15.00	562.00	6.29	
	14-732	8.45	17.30	49.10	58.00	20.40	35.20	16.10	1024.00	4.60	
	14-733	8.02	16.50	45.60	56.80	20.60	36.20	14.90	1369.00	5.36	
	Mean	**8.12**	**16.53**	**46.81**	**57.67**	**20.35**	**35.32**	**15.32**	**1078.80**	**5.23**	
	S.D.	**0.62**	**0.84**	**2.66**	**1.74**	**0.67**	**0.67**	**0.96**	**124.45**	**0.49**	
	14-692	8.61	17.90	50.70	58.90	20.80	35.30	14.60	1095.00	5.47	
100 mg/kg	14-693	9.53	18.80	54.50	57.10	19.70	34.50	16.80	1363.00	4.54	
	14-702	8.22	16.70	48.70	59.20	20.30	34.40	16.10	1087.00	5.98	
	14-703	7.96	16.60	47.60	59.80	20.90	35.00	14.80	1074.00	4.78	
	14-714	7.59	16.50	45.20	59.50	21.70	36.40	15.00	1249.00	5.81	
	14-715	8.98	17.60	48.90	54.50	19.60	35.90	16.80	1339.00	5.06	
	14-740	8.28	17.10	48.60	58.70	20.70	35.20	16.50	1303.00	4.78	
	14-741	8.33	17.30	48.30	58.00	20.80	35.90	15.60	1049.00	5.46	
	14-742	8.19	17.50	48.60	59.40	21.30	35.90	14.80	1060.00	5.39	
	14-743	7.45	15.80	45.20	60.70	21.20	34.90	14.30	1132.00	4.80	
	Mean	**8.31**	**17.18**	**48.63**	**58.58**	**20.70**	**35.34**	**15.53**	**1175.10**	**5.21**	
	S.D.	**0.46**	**0.89**	**2.58**	**1.04**	**0.33**	**0.37**	**0.40**	**232.58**	**0.48**	
	14-690	8.17	17.10	49.20	60.20	20.90	34.70	16.40	1052.00	5.73	
210 mg/kg	14-691	8.94	17.50	50.50	56.50	19.60	34.80	15.50	1218.00	4.53	
	14-718	8.39	16.20	47.40	56.50	19.30	34.20	14.80	1059.00	4.85	
	14-719	7.70	16.00	45.10	58.50	20.80	35.50	14.20	1050.00	4.83	
	14-744	8.38	18.10	50.10	59.70	21.50	36.10	16.00	1278.00	5.59	
	14-745	7.88	16.80	47.10	59.80	21.30	35.60	14.80	933.00	5.16	
	14-746	8.90	18.10	51.70	58.10	20.30	35.00	16.90	608.00	5.52	
	14-747	8.82	17.90	50.00	56.70	20.30	35.80	15.80	695.00	5.07	
	14-748	8.21	17.40	48.30	58.80	21.20	36.00	15.70	995.00	4.87	
	14-749	7.72	16.00	44.90	58.10	20.70	35.70	16.20	1169.00	4.94	
	Mean	**8.31**	**17.11**	**48.43**	**58.29**	**20.59**	**35.34**	**15.63**	**1005.70**	**5.11**	
	S.D.	**0.47**	**0.83**	**2.29**	**1.38**	**0.72**	**0.63**	**0.83**	**214.20**	**0.39**	
	14-708	7.94	17.20	49.00	61.70	21.70	35.20	15.90	1311.00	4.79	
415 mg/kg	14-709	7.52	15.80	44.70	59.40	21.10	35.50	14.80	1196.00	4.95	
	14-710	7.42	14.40	41.20	55.60	19.40	34.80	15.50	1305.00	5.20	
	14-711	8.64	17.60	49.80	57.60	20.30	35.30	16.00	1032.00	4.67	
	14-720	7.75	17.20	47.70	61.60	22.10	36.00	15.60	905.00	5.37	
	14-721	7.49	15.50	42.80	57.20	20.70	36.60	14.60	1211.00	5.01	
	14-722	7.92	16.70	46.80	59.10	21.10	35.60	15.40	1121.00	5.60	
	14-723	8.40	18.30	52.00	61.90	21.80	35.20	15.10	1041.00	4.89	
	14-728	7.28	15.70	44.30	60.80	21.60	35.60	14.90	1170.00	4.92	
	14-729	8.06	16.40	46.30	57.50	20.40	35.40	15.80	1071.00	5.00	
	Mean	**7.84**	**16.48**	**46.46**	**59.24**	**21.02**	**35.52**	**15.36**	**1136.30**	**5.04**	
	S.D.	**0.44**	**1.16**	**3.31**	**2.22**	**0.83**	**0.49**	**0.49**	**127.90**	**0.28**	

Dose	Animal ID	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)	Notes
	14-698	7.94	15.10	42.90	54.00	19.00	35.20	14.90	1098.00	5.55	
830 mg/kg	14-699	8.63	17.10	48.50	56.20	19.80	35.20	15.40	1136.00	4.57	
	14-704	8.19	16.50	47.20	57.70	20.10	34.90	15.30	815.00	4.91	
	14-705	7.84	15.20	43.40	55.40	19.40	35.10	15.20	950.00	5.04	
	14-712	8.51	17.10	48.30	56.80	20.10	35.40	15.20	1211.00	4.56	
	14-713	7.78	16.50	48.30	62.00	21.20	34.20	14.30	1371.00	4.89	
	14-726	7.84	17.20	48.60	62.00	21.90	35.40	17.10	1221.00	4.49	
	14-727	9.28	19.30	54.20	58.40	20.80	35.60	15.70	1249.00	4.82	
	14-736	8.94	17.80	50.10	56.00	19.90	35.50	17.10	1422.00	4.97	
	14-737	7.21	15.60	43.30	60.00	21.70	36.10	15.00	1091.00	5.19	
	Mean	**8.22**	**16.74**	**47.48**	**57.85**	**20.39**	**35.26**	**15.52**	**1156.40**	**4.90**	
	S.D.	**0.62**	**1.27**	**3.51**	**2.74**	**0.97**	**0.49**	**0.91**	**182.45**	**0.32**	
	14-696	8.33	17.00	47.20	56.70	20.40	35.90	16.50	1387.00	5.18	
1250 mg/kg	14-697	8.20	15.90	45.60	55.70	19.40	34.80	16.10	1025.00	5.30	
	14-706	8.78	17.90	50.40	57.40	20.40	35.50	16.50	1061.00	3.95	
	14-707	7.71	16.00	45.60	59.10	20.80	35.20	13.90	1075.00	4.89	
	14-724	7.92	15.70	45.50	57.50	19.80	34.40	14.50	832.00	5.34	
	14-725	8.17	16.80	47.30	57.90	20.60	35.50	14.40	1204.00	5.12	
	14-734	8.43	17.30	48.70	57.70	20.50	35.50	18.30	1323.00	5.37	
	14-735	8.06	16.30	46.00	57.00	20.30	35.50	15.70	1408.00	5.62	
	14-738	7.91	16.80	46.90	59.30	21.20	35.80	15.60	963.00	5.46	
	14-739	7.95	15.40	43.70	55.00	19.40	35.20	17.10	1229.00	5.56	
	Mean	**8.15**	**16.51**	**46.69**	**57.33**	**20.28**	**35.33**	**15.86**	**1150.70**	**5.18**	
	S.D.	**0.31**	**0.78**	**1.88**	**1.34**	**0.58**	**0.45**	**1.35**	**190.64**	**0.48**	

APPENDIX J
SUMMARY OF 14-DAY PROTHROMBIN TIMES AND INDIVIDUAL DATA

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table J-1
14-Day Prothrombin Time Summary
Male Rats

			MeNQ in Corn Oil				
		Corn Oil Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Average	Mean	9.77	9.66	9.71	9.67	9.48	9.70
PT	S.D.	0.39	0.40	0.33	0.26	0.33	0.22
	N	7	10	10	10	10	10

Table J-2
14-Day Individual Prothrombin Time
Male Rats

Dose	Animal ID	PT (sec)	Notes
	14-635	9.5	
Corn Oil	14-639	10.15	
Control	14-640	9.85	
	14-657	ND	Sample clotted
	14-658	9.4	
	14-673	9.7	
	14-674	ND	Sample clotted
	14-687	10.4	
	14-688	9.4	
	Mean	**9.77**	
	S.D.	**0.39**	
	14-641	8.8	
100 mg/kg	14-642	9.55	
	14-645	9.45	
	14-646	10	
	14-653	9.65	
	14-654	9.4	
	14-663	9.95	
	14-664	10.25	
	14-677	9.7	
	14-678	9.8	
	Mean	**9.66**	
	S.D.	**0.40**	
	14-629	9.35	
210 mg/kg	14-630	9.4	
	14-649	10.3	
	14-650	9.4	
	14-665	9.85	
	14-666	10.1	
	14-675	9.55	
	14-676	9.5	
	14-683	9.65	
	14-684	9.95	
	Mean	**9.71**	
	S.D.	**0.33**	
	14-625	10.05	
415 mg/kg	14-626	9.7	
	14-633	9.9	
	14-634	9.85	
	14-643	9.85	
	14-644	9.45	
	14-667	9.6	
	14-668	9.65	
	14-681	9.35	
	14-682	9.25	
	Mean	**9.67**	

	S.D.	**0.26**	
Dose	**Animal ID**	**PT (sec)**	**Notes**
	14-655	8.95	
830 mg/kg	14-656	9.5	
	14-661	9.4	
	14-662	9.7	
	14-669	9.15	
	14-670	9.45	
	14-679	9.8	
	14-680	9.1	
	14-685	9.75	
	14-686	9.95	
	Mean	**9.48**	
	S.D.	**0.33**	
	14-627	9.5	
1250 mg/kg	14-628	9.5	
	14-631	9.45	
	14-632	10	
	14-637	9.7	
	14-638	9.65	
	14-651	9.8	
	14-652	10.1	
	14-671	9.5	
	14-672	9.8	
	Mean	**9.70**	
	S.D.	**0.22**	

Table J-3
14-Day Prothrombin Time Summary
Female Rats

			MeNQ in Corn Oil				
		Corn Oil Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Average	Mean	9.29	9.31	9.16	9.57	9.36	9.24
PT	S.D.	0.36	0.46	0.31	1.30	0.20	0.21
	N	10	10	10	10	10	10

Table J-4
Individual Prothrombin Time Data
Female Rats

Dose	Animal ID	PT (sec)	Notes
Corn Oil Control	14-694	9.9	
	14-695	9.65	
	14-700	9.1	
	14-701	9.1	
	14-716	9.45	
	14-717	9.4	
	14-730	8.65	
	14-731	9.05	
	14-732	9.5	
	14-733	9.1	
	Mean	**9.29**	
	S.D.	**0.36**	
100 mg/kg	14-692	9.55	
	14-693	9.6	
	14-702	9.55	
	14-703	9.65	
	14-714	8.5	
	14-715	9.2	
	14-740	9.85	
	14-741	9.6	
	14-742	8.75	
	14-743	8.85	
	Mean	**9.31**	
	S.D.	**0.46**	
210 mg/kg	14-690	8.95	
	14-691	9.5	
	14-718	9.5	
	14-719	9.2	
	14-744	9.45	
	14-745	8.85	
	14-746	8.7	
	14-747	8.8	
	14-748	9.3	
	14-749	9.3	
	Mean	**9.16**	
	S.D.	**0.31**	
415 mg/kg	14-708	9.7	
	14-709	9.2	
	14-710	9.5	
	14-711	9.2	
	14-720	13.15	
	14-721	9.3	
	14-722	8.6	
	14-723	8.65	
	14-728	9.1	
	14-729	9.25	
	Mean	**9.57**	

Dose	Animal ID	PT (sec)	Notes
	S.D.	**1.30**	
	14-698	9.7	
830 mg/kg	14-699	9.1	
	14-704	9.25	
	14-705	9.4	
	14-712	9.3	
	14-713	9.1	
	14-726	9.4	
	14-727	9.35	
	14-736	9.3	
	14-737	9.65	
	Mean	**9.36**	
	S.D.	**0.20**	
	14-696	9.05	
1250 mg/kg	14-697	9	
	14-706	9.25	
	14-707	9.25	
	14-724	9.6	
	14-725	9.3	
	14-734	9.05	
	14-735	9.1	
	14-738	9.2	
	14-739	9.55	
	Mean	**9.24**	
	S.D.	**0.21**	

APPENDIX K
SUMMARY OF 14-DAY ORGAN WEIGHTS AND WEIGHT RATIOS

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Table K-1
Summary Absolute Organ Weight
Male Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Body weight	Mean	374.3	376.9	361.26	370.45	387.17	362.11
	S.D.	27.3	27.11	20.5	25.24	25.66	23.96
	N	9	10	10	10	10	10
Adrenals	Mean	0.053	0.056	0.056	0.055	0.055	0.051
	S.D.	0.015	0.072	0.009	0.012	0.012	0.007
	N	9	10	10	10	10	10
Brain	Mean	2.13	2.14	2.2	2.11	2.19	2.11
	S.D.	0.118	0.086	0.098	0.089	0.055	0.083
	N	9	10	10	10	10	10
Heart	Mean	1.4	1.42	1.43	1.4	1.46	1.3
	S.D.	0.127	0.154	0.158	0.18	0.139	0.104
	N	9	10	10	10	10	10
Kidneys	Mean	2.71	2.58	2.63	2.5	2.74	2.72
	S.D.	0.311	0.308	0.179	0.293	0.218	0.276
	N	9	10	10	10	10	10
Epididymides	Mean	0.893	0.846	0.893	0.86	0.854	0.852
	S.D.	0.094	0.069	0.065	0.108	0.081	0.108
	N	9	10	10	10	10	10
Liver	Mean	13.73	13.24	12.73	13.45	14.39	13.26
	S.D.	1.82	2.02	1.31	1.62	0.89	1.8
	N	9	10	10	10	10	10
Spleen	Mean	0.766	0.726	0.727	0.738	0.806	0.771
	S.D.	0.103	0.138	0.091	0.126	0.174	0.233
	N	9	10	10	10	10	10
Testes	Mean	3.32	3.1	3.23	3.02	2.98	3.16
	S.D.	0.247	0.276	0.326	0.285	0.597	0.368
	N	9	10	10	10	10	10
Thymus	Mean	0.481	0.484	0.42	0.449	0.469	0.451
	S.D.	0.095	0.07	0.076	0.08	0.079	0.079
	N	9	10	10	9	10	10

Table K-2
Summary Organ weights as a percent of body weight
Male Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Adrenals	Mean	0.014	0.015	0.016	0.015	0.014	0.014
	S.D.	0.0041	0.0015	0.0023	0.0027	0.0029	0.0022
	N	9	10	10	10	10	10
Brain	Mean	0.57	0.568	0.607	0.571	0.578	0.586
	S.D.	0.031	0.042	0.0372	0.041	0.033	0.0438
	N	9	10	10	10	10	10
Heart	Mean	0.375	0.377	0.398	0.378	0.378	0.358
	S.D.	0.0406	0.0352	0.0474	0.0372	0.0216	0.0264
	N	9	10	10	10	10	10
Kidneys	Mean	0.722	0.686	0.73	0.675	0.708	0.075
	S.D.	0.053	0.074	0.048	0.069	0.051	0.057
	N	9	10	10	10	10	10
Epididymides	Mean	0.24	0.225	0.248	0.233	0.222	0.235
	S.D.	0.0351	0.0123	0.0253	0.0306	0.282	0.273
	N	9	10	10	10	10	10
Liver	Mean	3.66	3.5	3.52	3.63	3.73	3.65
	S.D.	0.354	0.386	0.221	0.286	0.271	0.282
	N	9	10	10	10	10	10
Spleen	Mean	0.205	0.193	2.01	0.199	0.211	0.211
	S.D.	0.0294	0.0379	0.0203	0.0263	0.0434	0.0494
	N	9	10	10	10	10	10
Testes	Mean	0.89	0.824	0.896	0.816	0.77	0.874
	S.D.	0.0963	0.0706	0.096	0.0773	0.156	0.0657
	N	9	10	10	10	10	10
Thymus	Mean	0.128	0.128	0.116	0.123	0.122	0.112
	S.D.	0.02	0.0165	0.0177	0.02	0.023	0.0155
	N	9	10	10	9	10	10

Table K-3
Summary organ weights as a percent of brain weight
Male Rats

Organ		Corn Oil Control	MeNQ in Corn Oil 100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Adrenals	Mean	2.59	2.6	2.58	2.6	2.51	2.43
	S.D.	0.718	0.35	0.437	0.56	0.588	0.302
	N	9	10	10	10	10	10
Heart	Mean	65.7	66.5	65.7	66.8	66.9	61.3
	S.D.	6.82	7.31	8.71	9.94	3.39	4.18
	N	9	10	10	10	10	10
Kidneys	Mean	127.3	120.9	120.3	119.2	125	128.8
	S.D.	15.16	13.67	7	17.15	10.36	13.7
	N	9	10	10	10	10	10
Epididymides	Mean	42.1	39.6	40.9	40.9	39	40.3
	S.D.	5.6	3.33	3.75	5.39	3.99	4.73
	N	9	10	10	10	10	10
Liver	Mean	645.3	620.7	582.5	641.3	657.2	628.8
	S.D.	85.83	97.1	64.3	82.5	41.1	89.8
	N	9	10	10	10	10	10
Spleen	Mean	36.08	34.09	33.32	35.07	37.28	36.55
	S.D.	5.49	6.92	4.67	6.1	8.2	11.36
	N	9	10	10	10	10	10
Testes	Mean	156.2	145.1	147.8	143.5	136	149.6
	S.D.	17.2	11.1	16.6	15.2	27.9	11.5
	N	9	10	10	10	10	10
Thymus	Mean	22.7	22.7	19.3	21.4	21.5	19.2
	S.D.	4058	3.34	3.7	4.15	3.98	2.5
	N	9	10	10	9	10	10

Table K-4
Individual Data Absolute Organ Weights
Male Rats

Dose Group	Animal ID	Body weight	Adrenals	Brain	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
	14-0639	376.70	0.06	2.10	0.78	1.33	2.50	12.30	0.61	3.01	0.48
Corn Oil	14-0640	427.50	0.05	2.26	0.84	1.44	3.33	17.05	0.90	3.64	0.65
Control	14-0687	370.70	0.07	2.25	0.83	1.63	2.55	13.93	0.72	3.33	0.42
	14-0688	368.90	0.07	2.11	0.98	1.52	2.63	13.11	0.73	3.30	0.45
	14-0635	397.70	0.06	2.10	0.80	1.43	3.14	16.44	0.86	3.15	0.60
	14-0673	328.90	0.05	1.87	0.94	1.42	2.64	13.58	0.86	3.67	0.50
	14-0674	362.90	0.06	2.10	1.02	1.29	2.56	11.91	0.63	3.27	0.38
	14-0657	356.70	0.02	2.22	0.84	1.21	2.39	12.68	0.83	2.99	0.36
	14-0658	378.60	0.04	2.18	1.01	1.33	2.63	12.56	0.76	3.48	0.49
	Mean	**374.29**	**0.06**	**2.13**	**0.89**	**1.40**	**2.71**	**13.73**	**0.77**	**3.32**	**0.48**
	S.D.	**27.26**	**0.01**	**0.12**	**0.09**	**0.13**	**0.31**	**1.82**	**0.10**	**0.25**	**0.09**
	14-0645	349.00	0.06	2.02	0.81	1.34	2.04	9.86	0.58	3.14	0.41
100 mg/kg-d	14-0646	370.20	0.06	2.10	0.80	1.45	2.81	13.24	0.93	3.18	0.60
	14-0663	420.60	0.06	2.06	0.89	1.48	2.78	15.97	0.87	3.04	0.54
	14-0664	368.80	0.05	2.17	0.74	1.17	2.28	10.75	0.56	3.05	0.47
	14-0653	341.70	0.04	2.10	0.81	1.35	2.72	13.17	0.89	2.86	0.47
	14-0654	341.70	0.06	2.14	0.77	1.28	2.33	12.16	0.66	2.66	0.41
	14-0641	403.00	0.06	2.16	0.91	1.36	2.38	16.19	0.81	3.23	0.52
	14-0642	386.70	0.05	2.25	0.92	1.70	2.63	12.84	0.62	3.69	0.40
	14-0677	394.30	0.06	2.07	0.94	1.62	2.80	14.38	0.65	2.90	0.46
	14-0678	393.00	0.07	2.29	0.89	1.44	3.04	13.87	0.69	3.24	0.56
	Mean	**376.90**	**0.06**	**2.14**	**0.85**	**1.42**	**2.58**	**13.24**	**0.73**	**3.10**	**0.49**
	S.D.	**27.11**	**0.01**	**0.09**	**0.07**	**0.15**	**0.31**	**2.02**	**0.14**	**0.28**	**0.07**
	14-0629	370.80	0.06	2.22	0.85	1.33	2.64	12.48	0.63	3.26	0.46
210 mg/kg-d	14-0630	360.30	0.06	2.19	0.97	1.40	2.76	11.90	0.77	3.62	0.44
	14-0675	339.60	0.05	2.31	0.87	1.31	2.86	10.34	0.57	3.16	0.39
	14-0676	349.50	0.07	2.13	0.88	1.84	2.66	12.38	0.79	3.22	0.44
	14-0649	351.90	0.04	2.24	1.00	1.38	2.40	12.71	0.78	3.17	0.39
	14-0650	340.30	0.05	2.00	0.91	1.37	2.40	12.32	0.73	3.50	0.45
	14-0683	375.70	0.06	2.31	0.89	1.35	2.71	14.24	0.77	3.62	0.45
	14-0684	345.40	0.05	2.10	0.91	1.39	2.39	12.36	0.66	2.65	0.29
	14-0665	405.30	0.06	2.15	0.88	1.57	2.84	15.13	0.88	3.34	0.56
	14-0666	373.80	0.06	2.26	0.77	1.40	2.67	13.42	0.69	2.76	0.33
	Mean	**361.26**	**0.06**	**2.19**	**0.89**	**1.43**	**2.63**	**12.73**	**0.73**	**3.23**	**0.42**
	S.D.	**20.50**	**0.01**	**0.10**	**0.06**	**0.16**	**0.18**	**1.31**	**0.09**	**0.33**	**0.08**
	14-0625	382.50	0.05	2.15	0.93	1.24	2.43	12.80	0.52	3.40	0.50
415 mg/kg-d	14-0626	380.10	0.04	2.11	1.02	1.22	2.43	13.96	0.76	3.30	0.41
	14-0667	359.30	0.04	2.21	0.83	1.28	2.09	12.25	0.77	2.84	0.46
	14-0668	343.60	0.05	1.98	0.87	1.39	2.74	13.83	0.65	3.10	0.36
	14-0633	345.80	0.06	2.04	0.83	1.45	2.45	12.61	0.68	2.77	0.48
	14-0634	372.00	0.06	2.00	0.96	1.52	2.79	12.74	0.71	3.29	0.61
	14-0681	378.50	0.05	2.12	0.72	1.45	2.50	14.51	0.78	3.06	0.46
	14-0682	350.30	0.06	2.10	0.67	1.29	2.11	11.14	0.76	2.45	0.35
	14-0643	362.70	0.05	2.27	0.94	1.33	2.44	13.88	0.72	3.05	0.41
	14-0644	429.70	0.08	2.08	0.82	1.83	3.03	17.11	1.02	2.92	0.54
	Mean	**370.45**	**0.05**	**2.11**	**0.86**	**1.40**	**2.50**	**13.48**	**0.74**	**3.02**	**0.44**
	S.D.	**25.24**	**0.01**	**0.09**	**0.11**	**0.18**	**0.29**	**1.62**	**0.13**	**0.29**	**0.08**

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Dose Group	Animal ID	Body weight	Adrenals	Brain	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
	14-0679	367.10	0.04	2.22	0.92	1.40	2.60	14.41	0.90	3.09	0.46
830 mg/kg-d	14-0680	386.90	0.05	2.16	0.81	1.49	2.89	13.63	0.99	3.07	0.45
	14-0655	398.80	0.08	2.17	0.88	1.55	2.83	14.87	1.18	3.52	0.58
	14-0656	436.80	0.06	2.19	0.81	1.71	2.91	15.12	0.79	3.27	0.56
	14-0661	385.10	0.04	2.22	0.66	1.29	2.59	14.34	0.76	1.34	0.41
	14-0662	372.60	0.05	2.19	0.88	1.36	2.60	16.14	0.76	3.12	0.39
	14-0669	340.80	0.05	2.07	0.91	1.30	2.47	13.05	0.65	3.05	0.59
	14-0670	396.20	0.04	2.29	0.90	1.40	2.46	13.43	0.57	3.15	0.46
	14-0685	379.70	0.06	2.22	0.84	1.55	3.07	14.34	0.73	2.91	0.41
	14-0686	407.70	0.07	2.19	0.94	1.61	2.96	14.62	0.82	3.23	0.38
	Mean	**387.17**	**0.05**	**2.19**	**0.85**	**1.46**	**2.74**	**14.39**	**0.82**	**2.98**	**0.47**
	S.D.	**25.66**	**0.01**	**0.06**	**0.08**	**0.14**	**0.22**	**0.89**	**0.17**	**0.60**	**0.08**
	14-0637	355.50	0.04	1.99	0.73	1.17	2.48	12.28	0.63	3.09	0.36
1250 mg/kd-d	14-0638	344.10	0.06	2.19	0.81	1.14	2.47	13.04	0.69	3.01	0.42
	14-0627	395.00	0.05	2.12	0.96	1.37	3.04	16.07	0.89	3.63	0.48
	14-0628	358.20	0.05	2.05	0.73	1.30	2.60	13.74	0.63	2.85	0.39
	14-0671	358.50	0.06	2.18	0.80	1.44	2.51	11.71	0.77	3.07	0.48
	14-0672	361.80	0.05	2.17	0.94	1.34	3.19	12.88	0.94	3.26	0.35
	14-0631	353.20	0.05	2.07	1.01	1.23	2.56	12.56	0.64	3.35	0.40
	14-0632	327.00	0.05	2.04	0.72	1.20	2.64	10.66	0.59	2.77	0.44
	14-0651	357.20	0.05	2.25	0.94	1.42	2.60	13.25	0.59	3.47	0.33
	14-0652	410.60	0.06	2.05	0.88	1.35	3.10	16.45	1.33	3.10	0.40
	Mean	**362.11**	**0.05**	**2.11**	**0.85**	**1.30**	**2.72**	**13.26**	**0.77**	**3.16**	**0.41**
	S.D.	**23.96**	**0.01**	**0.08**	**0.11**	**0.10**	**0.28**	**1.80**	**0.23**	**0.27**	**0.05**

Table K-5
Individual Organ Weight as a Percent of Body Weight Data
Male Rats

Dose Group	Animal ID	Adrenals	Brain	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
	14-0639	0.01	0.56	0.21	0.35	0.66	3.26	0.16	0.80	0.13
Corn Oil	14-0640	0.01	0.53	0.20	0.34	0.78	3.99	0.21	0.85	0.15
Control	14-0687	0.02	0.61	0.22	0.44	0.69	3.76	0.19	0.90	0.11
	14-0688	0.02	0.57	0.27	0.41	0.71	3.55	0.20	0.89	0.12
	14-0635	0.02	0.53	0.20	0.36	0.79	4.13	0.22	0.79	0.15
	14-0673	0.02	0.57	0.28	0.43	0.80	4.13	0.26	1.12	0.15
	14-0674	0.02	0.58	0.28	0.35	0.70	3.28	0.17	0.90	0.11
	14-0657	0.01	0.62	0.23	0.34	0.67	3.56	0.23	0.84	0.10
	14-0658	0.01	0.58	0.27	0.35	0.69	3.32	0.20	0.92	0.13
	Mean	**0.01**	**0.56**	**0.24**	**0.37**	**0.72**	**3.66**	**0.21**	**0.89**	**0.13**
	S.D.	**0.00**	**0.03**	**0.04**	**0.04**	**0.05**	**0.35**	**0.03**	**0.10**	**0.02**
	14-0645	0.02	0.58	0.23	0.38	0.59	2.83	0.17	0.90	0.12
100 mg/kg-d	14-0646	0.01	0.57	0.22	0.39	0.76	3.58	0.25	0.86	0.16
	14-0663	0.01	0.49	0.21	0.35	0.66	3.80	0.21	0.72	0.13
	14-0664	0.01	0.59	0.20	0.32	0.62	2.91	0.15	0.83	0.13
	14-0653	0.01	0.62	0.24	0.39	0.80	3.85	0.26	0.84	0.14
	14-0654	0.02	0.63	0.23	0.37	0.68	3.56	0.19	0.78	0.12
	14-0641	0.01	0.54	0.23	0.34	0.59	4.02	0.20	0.80	0.13
	14-0642	0.01	0.58	0.24	0.44	0.68	3.32	0.16	0.95	0.10
	14-0677	0.02	0.52	0.24	0.41	0.71	3.65	0.16	0.74	0.12
	14-0678	0.02	0.58	0.23	0.37	0.77	3.53	0.17	0.82	0.14
	Mean	**0.01**	**0.57**	**0.22**	**0.38**	**0.70**	**3.58**	**0.20**	**0.82**	**0.13**
	S.D.	**0.00**	**0.04**	**0.01**	**0.04**	**0.07**	**0.32**	**0.04**	**0.07**	**0.02**
	14-0629	0.02	0.60	0.23	0.36	0.71	3.37	0.17	0.88	0.12
210 mg/kg-d	14-0630	0.02	0.61	0.27	0.39	0.77	3.30	0.21	1.00	0.12
	14-0675	0.01	0.68	0.26	0.39	0.84	3.05	0.17	0.93	0.11
	14-0676	0.02	0.61	0.25	0.53	0.76	3.54	0.23	0.92	0.13
	14-0649	0.01	0.64	0.29	0.39	0.68	3.61	0.22	0.90	0.11
	14-0650	0.02	0.59	0.27	0.40	0.71	3.62	0.21	1.03	0.13
	14-0683	0.02	0.61	0.24	0.36	0.72	3.79	0.21	0.96	0.12
	14-0684	0.01	0.61	0.26	0.40	0.69	3.58	0.19	0.77	0.08
	14-0665	0.02	0.53	0.22	0.39	0.70	3.73	0.22	0.82	0.14
	14-0666	0.02	0.60	0.20	0.37	0.71	3.59	0.18	0.74	0.09
	Mean	**0.02**	**0.61**	**0.25**	**0.40**	**0.73**	**3.53**	**0.20**	**0.90**	**0.12**
	S.D.	**0.00**	**0.04**	**0.03**	**0.05**	**0.05**	**0.23**	**0.02**	**0.10**	**0.02**
	14-0625	0.01	0.56	0.24	0.32	0.64	3.35	0.14	0.89	0.13
415 mg/kg-d	14-0626	0.01	0.55	0.27	0.32	0.64	3.67	0.20	0.87	0.11
	14-0667	0.01	0.61	0.23	0.36	0.58	3.41	0.22	0.79	0.13
	14-0668	0.02	0.58	0.25	0.41	0.80	4.03	0.19	0.90	0.10
	14-0633	0.02	0.59	0.24	0.42	0.71	3.65	0.20	0.80	0.14
	14-0634	0.02	0.54	0.26	0.41	0.75	3.42	0.19	0.88	0.16
	14-0681	0.01	0.56	0.19	0.38	0.66	3.83	0.21	0.81	0.12
	14-0682	0.02	0.60	0.19	0.37	0.60	3.18	0.22	0.70	0.10
	14-0643	0.01	0.63	0.26	0.37	0.67	3.83	0.20	0.84	0.11
	14-0644	0.02	0.48	0.19	0.43	0.71	3.98	0.24	0.68	0.13
	Mean	**0.01**	**0.57**	**0.23**	**0.38**	**0.68**	**3.67**	**0.21**	**0.81**	**0.12**
	S.D.	**0.00**	**0.04**	**0.03**	**0.03**	**0.07**	**0.28**	**0.02**	**0.08**	**0.02**

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Dose Group	Animal ID	Adrenals	Brain	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
	14-04679	0.01	0.60	0.25	0.38	0.71	3.93	0.25	0.84	0.13
830 mg/kg-d	14-0680	0.01	0.56	0.21	0.39	0.75	3.52	0.26	0.79	0.12
	14-0655	0.02	0.54	0.22	0.39	0.71	3.73	0.30	0.88	0.14
	14-0656	0.01	0.50	0.19	0.39	0.67	3.46	0.18	0.75	0.13
	14-0661	0.01	0.58	0.17	0.33	0.67	3.72	0.20	0.35	0.11
	14-0662	0.01	0.59	0.24	0.37	0.70	4.33	0.20	0.84	0.10
	14-0669	0.01	0.61	0.27	0.38	0.73	3.83	0.19	0.90	0.17
	14-0670	0.01	0.58	0.23	0.35	0.62	3.39	0.14	0.79	0.12
	14-0685	0.02	0.58	0.22	0.41	0.81	3.78	0.19	0.77	0.11
	14-0686	0.02	0.54	0.23	0.39	0.73	3.59	0.20	0.79	0.09
	Mean	**0.01**	**0.57**	**0.22**	**0.38**	**0.71**	**3.71**	**0.21**	**0.76**	**0.12**
	S.D.	**0.00**	**0.03**	**0.03**	**0.02**	**0.05**	**0.28**	**0.04**	**0.16**	**0.02**
	14-0637	0.01	0.56	0.21	0.33	0.70	3.46	0.18	0.87	0.10
1250 mg/kd-d	14-0638	0.02	0.64	0.23	0.33	0.72	3.79	0.20	0.87	0.12
	14-0627	0.01	0.54	0.24	0.35	0.77	4.07	0.23	0.92	0.12
	14-0628	0.01	0.57	0.20	0.36	0.73	3.84	0.17	0.80	0.11
	14-0671	0.02	0.61	0.22	0.40	0.70	3.27	0.21	0.86	0.13
	14-0672	0.01	0.60	0.26	0.37	0.88	3.56	0.26	0.90	0.10
	14-0631	0.01	0.59	0.29	0.35	0.73	3.56	0.18	0.95	0.11
	14-0632	0.01	0.62	0.22	0.37	0.81	3.26	0.18	0.85	0.14
	14-0651	0.01	0.63	0.26	0.40	0.73	3.71	0.16	0.97	0.09
	14-0652	0.01	0.50	0.21	0.33	0.75	4.01	0.32	0.76	0.10
	Mean	**0.01**	**0.59**	**0.24**	**0.36**	**0.76**	**3.67**	**0.21**	**0.87**	**0.11**
	S.D.	**0.00**	**0.04**	**0.03**	**0.03**	**0.06**	**0.29**	**0.05**	**0.07**	**0.02**

Table K-6
Individual Organ Weight as a Percent of Brain Weight Data
Male Rats

Dose Group	Animal ID	Adrenals	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
	14-0639	2.62	36.99	63.16	119.21	586.03	29.17	143.57	22.74
Corn Oil	14-0640	2.08	37.31	63.71	147.36	755.34	39.92	161.36	28.80
Control	14-0687	3.03	37.06	72.65	113.36	620.27	31.94	148.46	18.84
	14-0688	3.23	46.58	72.03	124.79	622.46	34.62	156.60	21.51
	14-0635	3.00	38.13	67.97	149.48	783.65	41.18	149.90	28.41
	14-0673	2.67	50.11	75.67	141.28	726.20	45.72	196.36	26.79
	14-0674	2.95	48.57	61.12	121.53	566.11	29.99	155.32	18.20
	14-0657	1.04	37.80	54.58	107.89	571.99	37.30	135.05	16.01
	14-0658	1.79	46.15	60.77	120.39	575.80	34.92	159.26	22.59
	Mean	**2.67**	**42.08**	**65.74**	**127.26**	**645.32**	**36.08**	**156.21**	**22.66**
	S.D.	**0.50**	**5.60**	**6.82**	**15.06**	**85.83**	**5.49**	**17.16**	**4.58**
	14-0645	2.73	39.98	66.52	101.34	489.19	28.72	155.80	20.09
100 mg/kg-d	14-0646	2.63	38.23	69.36	134.03	631.89	44.53	151.69	28.78
	14-0663	2.96	43.04	72.00	134.89	774.96	42.31	147.36	26.35
	14-0664	2.16	33.93	54.01	105.16	494.75	25.83	140.24	21.41
	14-0653	2.00	38.36	64.12	129.33	626.00	42.25	135.98	22.53
	14-0654	2.57	35.99	59.85	109.03	568.93	30.74	124.52	19.14
	14-0641	2.74	42.14	63.14	110.38	750.67	37.69	149.51	24.15
	14-0642	2.31	40.67	75.33	116.98	570.84	27.69	164.04	17.82
	14-0677	3.09	45.17	78.02	135.31	694.54	31.16	140.19	22.22
	14-0678	2.84	38.93	62.68	132.65	605.19	29.94	141.34	24.36
	Mean	**2.60**	**39.64**	**66.50**	**120.91**	**620.70**	**34.09**	**145.07**	**22.97**
	S.D.	**0.35**	**3.33**	**7.75**	**13.67**	**97.10**	**6.92**	**11.11**	**3.41**
	14-0629	2.57	38.45	59.84	118.78	562.09	28.28	146.83	20.76
210 mg/kg-d	14-0630	2.52	44.33	64.04	126.26	544.19	35.13	165.51	20.17
	14-0675	2.12	37.67	56.83	124.06	448.37	24.88	136.89	16.86
	14-0676	3.39	41.39	86.36	125.31	582.08	37.30	151.32	20.70
	14-0649	1.78	44.78	61.69	107.23	566.95	34.70	141.17	17.57
	14-0650	2.65	45.65	68.62	120.12	616.77	36.44	175.33	22.37
	14-0683	2.77	38.53	58.65	117.30	617.12	33.55	157.04	19.51
	14-0684	2.43	43.32	66.22	113.84	589.84	31.39	126.38	13.98
	14-0665	2.97	40.99	72.86	132.06	703.07	40.94	154.97	26.21
	14-0666	2.62	33.91	61.97	118.13	594.95	30.63	122.43	14.41
	Mean	**2.58**	**40.90**	**65.71**	**120.31**	**582.54**	**33.32**	**147.79**	**19.09**
	S.D.	**0.44**	**3.75**	**8.71**	**7.01**	**64.32**	**4.67**	**16.62**	**3.88**
	14-0625	2.28	43.50	57.95	113.24	596.64	24.24	158.41	23.40
415 mg/kg-d	14-0626	2.09	48.15	58.06	115.04	662.00	36.05	156.69	19.50
	14-0667	1.63	37.66	57.94	94.39	554.46	34.99	128.56	20.73
	14-0668	2.73	44.14	70.48	138.68	699.34	33.01	156.47	18.05
	14-0633	2.74	40.56	71.09	119.81	616.78	33.41	135.67	23.58
	14-0634	3.00	47.88	76.09	139.49	636.00	35.40	164.05	30.50
	14-0681	2.40	33.99	68.41	118.06	684.30	36.59	144.46	21.50
	14-0682	2.91	31.84	61.34	100.67	531.12	36.22	116.73	16.83
	14-0643	2.38	41.54	58.68	107.27	611.54	31.76	134.14	18.24
	14-0644	3.84	39.46	87.76	145.66	821.27	49.02	139.94	25.83
	Mean	**2.60**	**40.87**	**66.78**	**119.23**	**641.35**	**35.07**	**143.51**	**21.12**
	S.D.	**0.60**	**5.39**	**9.94**	**17.15**	**82.48**	**6.10**	**15.24**	**4.36**

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Dose Group	Animal ID	Adrenals	Epididymides	Heart	Kidneys	Liver	Spleen	Testes	Thymus
	14-0679	1.94	41.36	63.15	117.14	650.02	40.73	139.51	20.84
830 mg/kg-d	14-0680	2.46	37.38	69.11	133.86	632.37	46.01	142.35	20.96
	14-0655	3.78	40.47	71.34	130.55	686.39	54.41	162.21	26.63
	14-0656	2.79	37.05	77.98	132.80	690.91	36.14	149.52	25.67
	14-0661	1.89	29.68	57.91	116.64	644.60	34.35	60.21	18.35
	14-0662	2.19	40.15	62.28	118.70	737.86	34.66	142.84	17.83
	14-0669	2.46	43.90	62.47	119.20	629.33	31.45	147.18	28.41
	14-0670	1.92	39.22	61.17	107.39	587.23	24.88	137.60	20.07
	14-0685	2.70	37.81	69.90	138.26	646.10	32.94	130.96	18.66
	14-0686	2.97	42.97	73.42	135.25	667.58	37.26	147.63	17.40
	Mean	**2.51**	**39.00**	**66.87**	**124.98**	**657.24**	**37.28**	**136.00**	**21.55**
	S.D.	**0.59**	**3.99**	**6.39**	**10.36**	**41.13**	**8.20**	**27.89**	**4.21**
	14-0637	1.91	36.87	58.71	124.31	616.93	31.84	155.25	17.93
1250 mg/kd-d	14-0638	2.88	36.79	52.24	112.80	596.07	31.63	137.52	19.29
	14-0627	2.17	45.29	64.56	143.12	757.40	42.04	171.02	22.67
	14-0628	2.34	35.35	63.48	126.96	670.01	30.47	139.15	19.02
	14-0671	2.70	36.76	65.86	114.94	536.53	35.24	140.83	21.81
	14-0672	2.44	43.25	61.55	146.93	593.73	43.48	150.07	16.18
	14-0631	2.51	48.87	59.09	123.69	605.84	31.07	161.65	19.05
	14-0632	2.35	35.34	58.88	129.12	521.68	28.83	135.54	21.73
	14-0651	2.18	41.58	63.04	115.55	588.54	26.12	154.15	14.66
	14-0652	**2.78**	42.96	65.61	150.75	801.27	64.83	151.10	19.48
	Mean	**2.43**	**40.30**	**61.30**	**128.82**	**628.80**	**36.55**	**149.63**	**19.32**
	S.D.	**0.30**	**4.73**	**4.18**	**13.70**	**89.79**	**11.36**	**11.49**	**2.62**

Table K-7
Summary Data Absolute Organ Weights
Female Rats

Organ		Corn Oil Control	MeNQ in Corn Oil				
			100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Body weight	Mean	236.59	241.18	236.97	252.73	246.90	240.45
	S.D.	17.95	18.59	20.71	19.76	15.51	19.21
	N	10	10	10	10	10	10
Adrenals	Mean	0.06	0.06	0.07	0.07	0.07	0.06
	S.D.	0.01	0.01	0.01	0.01	0.02	0.01
	N	10	10	10	10	10	10
Brain	Mean	2.01	2.02	46.29	48.76	0.11	1.96
	S.D.	0.01	0.09	93.73	100.20	0.04	0.07
	N	10	10	10	10	10	10
Heart	Mean	0.91	1.01	0.99	1.00	0.98	0.98
	S.D.	0.10	0.11	0.16	0.06	0.08	0.14
	N	10	10	10	10	10	10
Kidneys	Mean	1.71	1.76	1.75	1.77	1.80	1.78
	S.D.	0.22	0.23	0.30	0.11	0.21	0.22
	N	10	10	10	10	10	10
Liver	Mean	7.95	8.61	8.39	8.99	8.67	8.27
	S.D.	1.19	0.97	1.14	0.90	0.73	1.05
	N	10	10	10	10	10	10
Ovaries	Mean	0.13	0.12	0.12	0.13	0.12	0.13
	S.D.	0.01	0.01	0.02	0.01	0.01	0.02
	N	10	10	10	10	10	10
Spleen	Mean	0.49	0.54	0.57	0.56	0.55	0.59
	S.D.	0.05	0.09	0.09	0.04	0.08	0.15
	N	10	10	10	10	10	10
Thymus	Mean	0.46	0.54	0.48	0.56	0.47	0.41[a]
	S.D.	0.08	0.09	0.13	0.07	0.12	0.10
	N	10	10	10	10	10	10
Uterus	Mean	0.60	0.52	0.52	0.53	0.55	0.78
	S.D.	0.26	0.11	0.16	0.20	0.21	0.32
	N	10	10	10	10	10	10

a. Significantly different from 415 mg/kg dose group, P = 0.023.

Table K-8
Summary Data Organ Weights as Percent Bodyweight
Female Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Adrenals	Mean	0.03	0.02	0.03	0.03	0.03	0.03
	S.D.	0.00	0.00	0.00	0.00	0.01	0.00
	N	10	10	10	10	10	10
Brain	Mean	0.85	0.84	0.85	0.80	0.82	0.82
	S.D.	0.06	0.06	0.07	0.07	0.07	0.07
	N	10	10	10	10	10	10
Heart	Mean	0.39	0.42	0.42	0.40	0.40	0.41
	S.D.	0.02	0.02	0.06	0.03	0.03	0.04
	N	10	10	10	10	10	10
Kidneys	Mean	0.72	0.73	0.73	0.70	0.73	0.74
	S.D.	0.06	0.08	0.08	0.04	0.07	0.05
	N	10	10	10	10	10	10
Liver	Mean	3.35	3.57	3.53	3.56	3.52	3.43
	S.D.	0.32	0.21	0.23	0.23	0.23	0.25
	N	10	10	10	10	10	10
Ovaries	Mean	0.05	0.05	0.05	0.05	0.05	0.05
	S.D.	0.01	0.00	0.00	0.00	0.00	0.01
	N	10	10	10	10	10	10
Spleen	Mean	0.21	0.22	0.24	0.22	0.22	0.24
	S.D.	0.03	0.04	0.03	0.01	0.02	0.05
	N	10	10	10	10	10	10
Thymus	Mean	0.19	0.22	0.20	0.22	0.19	0.17
	S.D.	0.02	0.03	0.04	0.03	0.05	0.04
	N	10	10	10	10	10	10
Uterus	Mean	0.26	0.22	0.22	0.21	0.22	0.33
	S.D.	0.12	0.05	0.06	0.08	0.08	0.13
	N	10	10	10	10	10	10

Table K-9
Summary Data Organ Weights as Percent Brain Weight
Female Rats

		Corn Oil	MeNQ in Corn Oil				
		Control	100 mg/kg	210 mg/kg	415 mg/kg	830 mg/kg	1250 mg/kg
Adrenals	Mean	3.10	2.97	3.28	3.42	3.33	3.30
	S.D.	0.42	0.46	0.34	0.43	0.93	0.60
	N	10	10	10	10	10	10
Heart	Mean	45.41	50.16	49.76	49.53	48.99	50.01
	S.D.	4.90	4.49	9.51	2.88	5.02	7.07
	N	10	10	10	10	10	10
Kidneys	Mean	85.17	87.40	87.31	88.18	89.59	90.63
	S.D.	10.95	11.46	13.24	6.64	11.62	10.55
	N	10	10	10	10	10	10
Liver	Mean	394.51	426.62	419.83	447.09	431.82	421.04
	S.D.	55.69	38.23	50.99	46.32	32.22	52.15
	N	10	10	10	10	10	10
Ovaries	Mean	6.39	5.79	6.08	6.30	6.21	6.56
	S.D.	0.50	0.62	0.70	0.68	0.67	0.94
	N	10	10	10	10	10	10
Spleen	Mean	24.33	26.59	28.68	28.06	27.41	30.21
	S.D.	2.94	4.75	4.47	2.58	4.32	7.49
	N	10	10	10	10	10	10
Thymus	Mean	22.76	26.71	24.06	27.88	23.23	21.01
	S.D.	3.90	4.15	6.79	3.98	6.12	5.17
	N	10	10	10	10	10	10
Uterus	Mean	29.93	25.97	26.18	26.51	27.67	39.93
	S.D.	12.44	6.19	7.58	10.25	11.41	16.78
	N	10	10	10	10	10	10

Table K-10
Absolute Organ Weight Individual Data
Female Rats

Dose Group	Animal ID	Body weight	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	14-0694	235.30	0.07	1.98	0.90	1.64	8.14	0.14	0.52	0.49	0.40
Corn Oil	14-0695	221.70	0.06	1.96	0.85	1.78	8.03	0.13	0.50	0.33	0.89
Control	14-0700	243.10	0.06	2.05	0.85	1.80	8.43	0.15	0.46	0.44	0.45
	14-0701	208.70	0.06	2.01	0.77	1.38	5.73	0.14	0.44	0.39	0.47
	14-0716	229.20	0.06	1.93	0.93	1.48	7.73	0.12	0.58	0.44	0.74
	14-0717	225.60	0.06	2.10	0.81	1.59	7.29	0.12	0.48	0.40	1.17
	14-0730	254.40	0.08	1.98	1.03	2.14	9.86	0.12	0.46	0.57	0.62
	14-0731	273.50	0.07	2.22	1.06	1.90	9.60	0.12	0.50	0.52	0.43
	14-0732	237.70	0.05	1.97	0.99	1.63	7.37	0.13	0.55	0.55	0.43
	14-0733	236.70	0.05	1.93	0.95	1.82	7.29	0.12	0.41	0.47	0.44
	Mean	**236.59**	**0.06**	**2.01**	**0.91**	**1.71**	**7.95**	**0.13**	**0.49**	**0.46**	**0.60**
	S.D.	**17.95**	**0.01**	**0.09**	**0.10**	**0.22**	**1.19**	**0.01**	**0.05**	**0.08**	**0.26**
	14-0692	217.50	0.04	1.91	0.83	1.33	7.36	0.11	0.45	0.53	0.54
100 mg/kg-d	14-0693	258.80	0.06	2.06	1.11	1.83	9.93	0.12	0.49	0.52	0.43
	14-0702	231.90	0.06	1.90	1.02	2.06	8.10	0.10	0.47	0.42	0.77
	14-0703	229.00	0.06	1.93	0.88	1.77	7.88	0.12	0.74	0.38	0.44
	14-0714	254.30	0.07	2.01	1.09	1.80	9.62	0.12	0.56	0.57	0.66
	14-0715	212.90	0.06	2.09	0.88	1.50	7.79	0.11	0.45	0.53	0.46
	14-0740	270.80	0.08	2.16	1.14	2.13	10.12	0.11	0.58	0.59	0.50
	14-0741	242.20	0.06	1.96	1.03	1.74	8.39	0.14	0.55	0.69	0.46
	14-0742	240.80	0.07	2.11	1.09	1.77	8.88	0.11	0.58	0.58	0.50
	14-0743	253.60	0.06	2.03	1.05	1.68	8.04	0.13	0.48	0.59	0.45
	Mean	**241.18**	**0.06**	**2.02**	**1.01**	**1.76**	**8.61**	**0.12**	**0.54**	**0.54**	**0.52**
	S.D.	**18.59**	**0.01**	**0.09**	**0.11**	**0.23**	**0.97**	**0.01**	**0.09**	**0.09**	**0.11**
	14-0690	232.70	0.06	1.99	0.98	1.68	8.08	0.12	0.51	0.43	0.51
210 mg/kg-d	14-0691	214.00	0.06	1.97	0.75	1.57	7.35	0.09	0.50	0.29	0.74
	14-0718	215.70	0.06	2.02	0.86	1.46	6.99	0.12	0.44	0.30	0.40
	14-0719	226.20	0.07	1.99	0.98	1.49	7.67	0.12	0.53	0.50	0.42
	14-0744	281.60	0.08	2.07	1.06	2.41	10.88	0.15	0.72	0.65	0.87
	14-0745	229.30	0.08	2.06	0.93	2.01	9.05	0.13	0.65	0.38	0.50
	14-0746	230.10	0.07	2.04	0.89	1.63	8.30	0.12	0.54	0.45	0.50
	14-0747	232.20	0.05	1.85	1.34	1.50	7.91	0.11	0.60	0.55	0.44
	14-0748	249.00	0.07	1.97	1.14	1.81	8.18	0.12	0.53	0.56	0.44
	14-0749	258.90	0.06	2.00	0.96	1.91	9.44	0.14	0.71	0.69	0.42
	Mean	**236.97**	**0.07**	**2.00**	**0.99**	**1.75**	**8.39**	**0.12**	**0.57**	**0.48**	**0.52**
	S.D.	**20.71**	**0.01**	**0.06**	**0.16**	**0.30**	**1.14**	**0.02**	**0.09**	**0.13**	**0.16**
	14-0708	288.10	0.08	2.00	1.11	1.99	10.03	0.13	0.61	0.55	0.69
415 mg/kg-d	14-0709	266.00	0.07	2.03	0.97	1.72	9.65	0.13	0.62	0.65	0.62
	14-0710	248.40	0.07	2.02	0.97	1.68	7.96	0.15	0.52	0.45	1.00
	14-0711	214.70	0.06	2.03	0.95	1.62	7.92	0.11	0.53	0.50	0.32
	14-0720	269.40	0.08	2.12	1.01	1.84	10.50	0.14	0.62	0.62	0.38
	14-0721	236.00	0.06	1.93	1.00	1.83	8.66	0.12	0.55	0.58	0.46
	14-0722	245.00	0.08	2.00	0.96	1.79	8.73	0.11	0.51	0.63	0.39
	14-0723	254.30	0.07	2.17	1.06	1.73	8.13	0.13	0.54	0.46	0.42
	14-0728	251.90	0.06	1.98	1.05	1.87	9.52	0.11	0.53	0.62	0.47
	14-0729	253.50	0.06	1.87	0.91	1.65	8.79	0.13	0.61	0.56	0.57
	Mean	**252.73**	**0.07**	**2.01**	**1.00**	**1.77**	**8.99**	**0.13**	**0.56**	**0.56**	**0.53**
	S.D.	**19.76**	**0.01**	**0.08**	**0.06**	**0.11**	**0.90**	**0.01**	**0.04**	**0.07**	**0.20**

Toxicology Study No. S.0024883, July-September 2014

Dose Group	Animal ID	Body weight	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	14-0698	251.30	0.09	1.86	1.11	2.12	7.63	0.12	0.49	0.44	0.95
830 mg/kg-d	14-0699	237.80	0.06	1.98	0.98	1.82	8.97	0.11	0.57	0.71	0.43
	14-0704	256.70	0.06	2.02	1.00	1.63	8.51	0.14	0.57	0.30	0.57
	14-0705	230.80	0.03	1.93	0.89	1.40	7.65	0.10	0.44	0.33	0.40
	14-0712	222.50	0.07	2.02	0.95	1.65	8.11	0.11	0.42	0.45	0.52
	14-0713	256.40	0.07	2.06	1.10	1.90	9.34	0.12	0.62	0.49	0.37
	14-0726	256.60	0.09	1.99	0.96	1.99	9.51	0.14	0.60	0.47	0.90
	14-0727	234.70	0.07	2.26	0.92	1.84	8.76	0.14	0.49	0.42	0.39
	14-0736	247.40	0.07	1.90	0.87	1.70	8.56	0.14	0.62	0.46	0.43
	14-0737	274.80	0.07	2.07	1.03	1.91	9.70	0.13	0.68	0.61	0.56
	Mean	**246.90**	**0.07**	**2.01**	**0.98**	**1.80**	**8.67**	**0.12**	**0.55**	**0.47**	**0.55**
	S.D.	**15.51**	**0.02**	**0.11**	**0.08**	**0.21**	**0.73**	**0.01**	**0.08**	**0.12**	**0.21**
	14-0696	249.00	0.07	1.98	1.06	1.85	9.38	0.13	0.48	0.45	0.57
1250 mg/kd-d	14-0697	196.80	0.05	1.91	0.75	1.31	6.64	0.11	0.39	0.21	0.66
	14-0706	254.10	0.06	1.95	1.25	1.69	8.49	0.15	0.58	0.47	0.42
	14-0707	225.20	0.05	2.07	0.90	1.59	7.29	0.10	0.42	0.40	0.94
	14-0724	264.70	0.06	1.99	1.04	1.89	9.26	0.15	0.81	0.36	1.24
	14-0725	241.30	0.07	1.95	0.96	1.78	7.12	0.12	0.47	0.46	0.86
	14-0734	242.10	0.06	2.00	0.91	1.89	8.32	0.13	0.71	0.34	0.46
	14-0735	243.80	0.06	1.86	1.05	1.85	8.95	0.14	0.70	0.49	0.93
	14-0738	256.00	0.07	2.05	1.06	2.13	9.63	0.12	0.71	0.59	0.45
	14-0739	231.50	0.09	1.88	0.85	1.80	7.59	0.14	0.65	0.37	1.26
	Mean	**240.45**	**0.06**	**1.96**	**0.98**	**1.78**	**8.27**	**0.13**	**0.59**	**0.41**	**0.78**
	S.D.	**19.21**	**0.01**	**0.07**	**0.14**	**0.22**	**1.05**	**0.02**	**0.15**	**0.10**	**0.32**

Table K-11
Organ Weights as Percent Bodyweight Individual Data
Female Rats

Dose Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	14-0694	0.030	0.840	0.382	0.697	3.458	0.058	0.221	0.206	0.168
Corn Oil	14-0695	0.027	0.886	0.382	0.802	3.623	0.058	0.226	0.147	0.401
Control	14-0700	0.023	0.843	0.348	0.738	3.466	0.060	0.188	0.181	0.186
	14-0701	0.029	0.965	0.369	0.662	2.743	0.065	0.213	0.186	0.227
	14-0716	0.026	0.843	0.406	0.644	3.372	0.052	0.253	0.191	0.322
	14-0717	0.027	0.933	0.359	0.703	3.232	0.055	0.213	0.175	0.517
	14-0730	0.031	0.780	0.406	0.841	3.875	0.046	0.179	0.224	0.243
	14-0731	0.026	0.810	0.388	0.693	3.512	0.045	0.182	0.189	0.155
	14-0732	0.022	0.827	0.416	0.686	3.100	0.054	0.230	0.231	0.181
	14-0733	0.023	0.817	0.399	0.767	3.080	0.052	0.171	0.198	0.185
	Mean	**0.026**	**0.854**	**0.386**	**0.723**	**3.346**	**0.055**	**0.208**	**0.193**	**0.258**
	S.D.	**0.003**	**0.057**	**0.022**	**0.063**	**0.320**	**0.006**	**0.026**	**0.024**	**0.119**
	14-0692	0.017	0.878	0.382	0.611	3.383	0.051	0.205	0.243	0.247
100 mg/kg-d	14-0693	0.024	0.796	0.428	0.707	3.837	0.047	0.190	0.202	0.165
	14-0702	0.024	0.821	0.439	0.889	3.494	0.044	0.203	0.183	0.331
	14-0703	0.025	0.841	0.386	0.774	3.440	0.051	0.324	0.165	0.191
	14-0714	0.026	0.792	0.428	0.707	3.783	0.047	0.219	0.224	0.261
	14-0715	0.026	0.981	0.415	0.705	3.661	0.050	0.213	0.247	0.217
	14-0740	0.028	0.799	0.421	0.786	3.736	0.040	0.213	0.217	0.183
	14-0741	0.024	0.808	0.424	0.720	3.464	0.056	0.229	0.283	0.192
	14-0742	0.031	0.876	0.453	0.733	3.686	0.047	0.241	0.240	0.209
	14-0743	0.024	0.799	0.415	0.662	3.172	0.052	0.189	0.234	0.177
	Mean	**0.025**	**0.839**	**0.419**	**0.730**	**3.565**	**0.048**	**0.223**	**0.224**	**0.217**
	S.D.	**0.004**	**0.059**	**0.022**	**0.075**	**0.209**	**0.005**	**0.039**	**0.034**	**0.050**
	14-0690	0.025	0.855	0.421	0.722	3.474	0.050	0.219	0.186	0.219
210 mg/kg-d	14-0691	0.029	0.919	0.352	0.733	3.436	0.044	0.233	0.136	0.347
	14-0718	0.027	0.938	0.398	0.677	3.239	0.054	0.205	0.140	0.185
	14-0719	0.030	0.878	0.434	0.659	3.391	0.053	0.234	0.220	0.186
	14-0744	0.028	0.735	0.377	0.855	3.864	0.055	0.255	0.229	0.310
	14-0745	0.034	0.899	0.406	0.877	3.945	0.055	0.281	0.167	0.219
	14-0746	0.029	0.885	0.386	0.707	3.608	0.053	0.236	0.195	0.216
	14-0747	0.023	0.798	0.576	0.644	3.407	0.047	0.258	0.236	0.188
	14-0748	0.027	0.790	0.456	0.726	3.286	0.047	0.211	0.226	0.176
	14-0749	0.025	0.774	0.372	0.738	3.645	0.053	0.274	0.265	0.161
	Mean	**0.028**	**0.847**	**0.418**	**0.734**	**3.530**	**0.051**	**0.241**	**0.200**	**0.221**
	S.D.	**0.003**	**0.069**	**0.063**	**0.077**	**0.234**	**0.004**	**0.026**	**0.043**	**0.061**
	14-0708	0.027	0.693	0.385	0.691	3.481	0.046	0.211	0.190	0.240
415 mg/kg-d	14-0709	0.024	0.763	0.364	0.646	3.626	0.050	0.232	0.242	0.232
	14-0710	0.029	0.812	0.390	0.677	3.205	0.058	0.209	0.179	0.404
	14-0711	0.026	0.946	0.440	0.756	3.687	0.051	0.245	0.232	0.150
	14-0720	0.031	0.785	0.375	0.681	3.898	0.053	0.230	0.231	0.139
	14-0721	0.026	0.819	0.422	0.777	3.669	0.053	0.234	0.244	0.195
	14-0722	0.033	0.814	0.391	0.729	3.563	0.043	0.209	0.256	0.158
	14-0723	0.027	0.852	0.415	0.681	3.197	0.051	0.212	0.179	0.164
	14-0728	0.024	0.784	0.417	0.742	3.779	0.045	0.212	0.247	0.187
	14-0729	0.025	0.739	0.357	0.652	3.469	0.052	0.240	0.221	0.224
	Mean	**0.027**	**0.801**	**0.396**	**0.703**	**3.557**	**0.050**	**0.224**	**0.222**	**0.209**
	S.D.	**0.003**	**0.068**	**0.027**	**0.045**	**0.228**	**0.005**	**0.014**	**0.029**	**0.077**

Toxicology Study No. S.0024883, July-September 2014

Dose Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	14-0698	0.035	0.740	0.442	0.842	3.036	0.049	0.197	0.173	0.376
830 mg/kg-d	14-0699	0.023	0.833	0.413	0.763	3.771	0.045	0.238	0.298	0.179
	14-0704	0.022	0.788	0.388	0.636	3.314	0.055	0.221	0.115	0.220
	14-0705	0.011	0.838	0.387	0.607	3.316	0.045	0.191	0.141	0.172
	14-0712	0.030	0.907	0.425	0.743	3.645	0.050	0.191	0.202	0.234
	14-0713	0.027	0.803	0.429	0.741	3.644	0.047	0.241	0.189	0.145
	14-0726	0.034	0.777	0.376	0.777	3.705	0.054	0.234	0.182	0.352
	14-0727	0.032	0.963	0.393	0.783	3.734	0.060	0.208	0.179	0.164
	14-0736	0.028	0.768	0.352	0.688	3.459	0.055	0.249	0.188	0.173
	14-0737	0.027	0.755	0.376	0.696	3.531	0.047	0.248	0.222	0.205
	Mean	**0.027**	**0.817**	**0.398**	**0.728**	**3.515**	**0.051**	**0.222**	**0.189**	**0.222**
	S.D.	**0.007**	**0.071**	**0.028**	**0.071**	**0.235**	**0.005**	**0.024**	**0.049**	**0.080**
	14-0696	0.030	0.796	0.425	0.744	3.767	0.053	0.191	0.182	0.228
1250 mg/kd-d	14-0697	0.027	0.968	0.382	0.668	3.374	0.057	0.199	0.104	0.337
	14-0706	0.024	0.769	0.490	0.665	3.342	0.059	0.227	0.184	0.164
	14-0707	0.024	0.919	0.400	0.707	3.239	0.044	0.187	0.179	0.419
	14-0724	0.024	0.750	0.391	0.714	3.498	0.055	0.306	0.134	0.469
	14-0725	0.029	0.809	0.396	0.739	2.950	0.050	0.195	0.189	0.356
	14-0734	0.026	0.828	0.374	0.782	3.437	0.052	0.294	0.140	0.189
	14-0735	0.023	0.761	0.431	0.758	3.669	0.056	0.288	0.202	0.383
	14-0738	0.026	0.802	0.414	0.832	3.760	0.046	0.278	0.229	0.175
	14-0739	0.038	0.811	0.368	0.778	3.277	0.062	0.282	0.162	0.546
	Mean	**0.027**	**0.821**	**0.407**	**0.739**	**3.431**	**0.053**	**0.245**	**0.170**	**0.327**
	S.D.	**0.005**	**0.070**	**0.036**	**0.052**	**0.255**	**0.005**	**0.049**	**0.036**	**0.133**

Table K-12
Organ Weight as Percent Brain Weight Individual Data
Female Rats

Dose Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	14-0694	3.591	45.524	82.954	411.583	6.879	26.353	24.532	19.980
Corn Oil	14-0695	3.004	43.126	90.580	409.012	6.568	25.509	16.599	45.265
Control	14-0700	2.780	41.317	87.561	411.073	7.171	22.341	21.463	22.000
	14-0701	2.981	38.251	68.654	284.401	6.756	22.057	19.275	23.497
	14-0716	3.106	48.188	76.398	400.000	6.211	30.021	22.619	38.251
	14-0717	2.852	38.498	75.333	346.578	5.894	22.861	18.774	55.418
	14-0730	4.032	52.117	107.813	496.825	5.897	22.984	28.780	31.149
	14-0731	3.251	47.901	85.598	433.589	5.553	22.438	23.386	19.187
	14-0732	2.646	50.331	82.952	375.013	6.565	27.786	27.990	21.883
	14-0733	2.792	48.862	93.847	376.991	6.360	20.941	24.199	22.647
	Mean	**3.104**	**45.412**	**85.169**	**394.507**	**6.385**	**24.329**	**22.762**	**29.928**
	S.D.	**0.424**	**4.899**	**10.952**	**55.688**	**0.502**	**2.943**	**3.900**	**12.436**
	14-0692	1.885	43.508	69.581	385.183	5.812	23.298	27.696	28.168
100 mg/kg-d	14-0693	3.008	53.712	88.840	481.756	5.871	23.872	25.328	20.718
	14-0702	2.941	53.519	108.298	425.525	5.305	24.685	22.269	40.284
	14-0703	2.960	45.846	92.056	408.982	6.075	38.577	19.574	22.741
	14-0714	3.277	54.022	89.225	477.607	5.958	27.656	28.252	32.969
	14-0715	2.681	42.269	71.853	373.097	5.074	21.685	25.132	22.116
	14-0740	3.467	52.705	98.428	467.776	5.039	26.676	27.138	22.931
	14-0741	2.964	52.427	89.116	428.666	6.898	28.309	35.003	23.710
	14-0742	3.509	51.731	83.736	420.816	5.311	27.549	27.454	23.850
	14-0743	3.009	51.899	82.832	396.793	6.561	23.631	29.304	22.200
	Mean	**2.970**	**50.164**	**87.397**	**426.620**	**5.790**	**26.594**	**26.715**	**25.969**
	S.D.	**0.459**	**4.485**	**11.460**	**38.232**	**0.622**	**4.754**	**4.145**	**6.188**
	14-0690	2.916	49.271	84.414	406.435	5.882	25.641	21.719	25.591
210 mg/kg-d	14-0691	3.152	38.282	79.766	373.818	4.779	25.369	14.794	37.722
	14-0718	2.867	42.462	72.219	345.329	5.783	21.898	14.928	19.674
	14-0719	3.375	49.421	75.063	386.448	6.096	26.700	25.038	21.159
	14-0744	3.867	51.329	116.336	525.906	7.443	34.703	31.223	42.243
	14-0745	3.734	45.199	97.575	438.700	6.159	31.280	18.526	24.345
	14-0746	3.289	43.643	79.823	407.609	6.038	26.706	22.042	24.448
	14-0747	2.914	72.153	80.680	426.983	5.882	32.326	29.520	23.583
	14-0748	3.459	57.731	91.913	416.124	5.900	26.704	28.586	22.228
	14-0749	3.194	48.104	95.359	470.958	6.886	35.429	34.182	20.808
	Mean	**3.277**	**49.759**	**87.315**	**419.831**	**6.085**	**28.676**	**24.056**	**26.180**
	S.D.	**0.342**	**9.509**	**13.237**	**50.988**	**0.700**	**4.470**	**6.786**	**7.577**
	14-0708	3.908	55.501	99.800	502.455	6.613	30.461	27.455	34.619
415 mg/kg-d	14-0709	3.204	47.659	84.672	475.407	6.555	30.458	31.789	30.458
	14-0710	3.571	48.065	83.383	394.841	7.192	25.794	22.073	49.802
	14-0711	2.708	46.529	79.911	389.710	5.416	25.849	24.569	15.903
	14-0720	3.924	47.801	86.761	496.454	6.761	29.314	29.409	17.730
	14-0721	3.156	51.526	94.827	447.905	6.415	28.608	29.747	23.849
	14-0722	4.010	48.020	89.574	437.544	5.263	25.664	31.378	19.449
	14-0723	3.184	48.731	79.926	375.173	5.999	24.919	20.997	19.197
	14-0728	3.089	53.165	94.684	481.975	5.722	27.038	31.443	23.797
	14-0729	3.417	48.318	88.254	469.461	7.101	32.515	29.899	30.326
	Mean	**3.417**	**49.532**	**88.179**	**447.092**	**6.304**	**28.062**	**27.876**	**26.513**
	S.D.	**0.428**	**2.884**	**6.644**	**46.317**	**0.676**	**2.580**	**3.977**	**10.251**

Toxicology Study No. S.0024883, July-September 2014

Dose Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
	14-0698	4.734	59.763	113.825	410.436	6.670	26.573	23.453	50.834
830 mg/kg-d	14-0699	2.778	49.646	91.667	452.929	5.404	28.586	35.808	21.465
	14-0704	2.819	49.258	80.712	420.722	6.924	28.091	14.590	27.943
	14-0705	1.344	46.174	72.441	395.708	5.377	22.751	16.805	20.527
	14-0712	3.320	46.829	81.962	401.933	5.500	21.011	22.299	25.818
	14-0713	3.401	53.450	92.323	454.033	5.879	30.078	23.567	18.124
	14-0726	4.415	48.369	100.050	476.969	6.924	30.156	23.432	45.309
	14-0727	3.273	40.823	81.247	387.572	6.192	21.628	18.532	17.028
	14-0736	3.682	45.765	89.479	450.132	7.102	32.404	24.408	22.567
	14-0737	3.568	49.807	92.237	467.792	6.172	32.835	29.412	27.097
	Mean	**3.333**	**48.988**	**89.594**	**431.823**	**6.214**	**27.411**	**23.231**	**27.671**
	S.D.	**0.934**	**5.025**	**11.625**	**32.219**	**0.665**	**4.316**	**6.118**	**11.409**
	14-0696	3.734	53.380	93.441	473.259	6.660	23.966	22.805	28.658
1250 mg/kd-d	14-0697	2.782	39.475	68.976	348.556	5.879	20.577	10.761	34.803
	14-0706	3.123	63.748	86.534	434.869	7.680	29.493	23.963	21.301
	14-0707	2.560	43.478	76.908	352.367	4.831	20.338	19.420	45.604
	14-0724	3.172	52.165	95.166	466.213	7.351	40.735	17.925	62.538
	14-0725	3.635	48.899	91.295	364.516	6.144	24.117	23.297	43.984
	14-0734	3.094	45.210	94.511	415.269	6.337	35.479	16.866	22.854
	14-0735	2.965	56.604	99.677	482.264	7.332	37.898	26.577	50.350
	14-0738	3.215	51.680	103.799	468.826	5.748	34.681	28.544	21.870
	14-0739	4.688	45.445	96.004	404.209	7.619	34.790	19.925	67.288
	Mean	**3.297**	**50.008**	**90.631**	**421.035**	**6.558**	**30.207**	**21.008**	**39.925**
	S.D.	**0.601**	**7.069**	**10.552**	**52.145**	**0.939**	**7.494**	**5.168**	**16.783**

APPENDIX L
HISTOPATHOLOGY REPORT

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Study Title

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus)*

Protocol No. 30-14-07-01

REPORT OF HISTOPATHOLOGY

Prepared by:

Erica Eggers Carroll, DVM, PhD, Diplomate ACVP

Final Approval Date:

16 December 2015

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This pathology investigation was conducted in a manner consistent with the principles of the United States Environmental Protection Agency (USEPA) Good Laboratory Practice regulations of the Toxic Substances Control Act (TSCA), as detailed in 40 CFR Part 792, plus amendments.

CARROLL.ERICA.E.1027432413

7 October 2015

Erica E. Carroll, DVM, PhD, Diplomate ACVP — Date

LTC, VC

Study Pathologist
Toxicology Portfolio

Army Public Health Center (Provisional)

QUALITY ASSURANCE STATEMENT

The following critical phases were audited by the APHC (Prov) Quality Systems and Regulatory Compliance Office (QSARC), Laboratory and Toxicology Accreditation and Compliance Office (LTACO):

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to
Interim Contributing Scientist Pathology Study Report and Raw Data Good Laboratory Practice	09/30/2015	10/07/2015
Quality Assurance audit of Statistician's report and Excel	10/01/2015	10/07/2015
Final Contributing Scientist Pathology Study Report Good Laboratory Practice Standards Review	10/06/2015	10/07/2015
Final Study Raw Data Good Laboratory Practice Standards	10/06/2015	10/07/2015

Note 1 All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2 In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedure.

Note 3 This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver
Quality Assurance Specialist, QSARC

07 OCT 2015

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (Rattus norvegicus) Protocol No. 30-14-07-01

1. INTRODUCTION

The purpose of this study is to assess the repeated dose oral toxicity of methylnitroguanidine (MeNQ), a component of a new mixture formulated to replace 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)-based fills in explosive formulations in an effort to reduce environmental and/or occupational health hazards. In this subacute oral toxicity study female and male rats were orally dosed with MeNQ at 1,250 milligrams per kilogram (mg/kg), 830 mg/kg, 415 mg/kg, 210 mg/kg, 100 mg/kg daily or 0 mg/kg (corn oil vehicle) for 14 consecutive days to determine any toxic effects of repeated exposures to the test material. Tissues were examined to determine evidence of toxicity. Refer to **Appendix A** for a list of references.

2. METHODS

According to the protocol and Toxicology Standard Operating Procedures (TOX SOP) 014 Pathology Laboratory Operations, TOX SOP 016 Histopathology Laboratory Operations, and TOX 076 General Embedding Procedures, rats were humanely sacrificed, investigator-specified tissues were formalin-fixed, paraffin-embedded, and all but the male reproductive tissues (which were fixed in modified Davidson's) were processed onto hematoxylin and eosin-stained microscope slides. Ten male high-dose (1,250 mg/kg) and medium-high dose (830 mg/kg) rats were evaluated and compared to nine vehicle-only (corn oil) control male rats. Ten high-dose (1,250 mg/kg/day) female rats were compared with 10 female control rats administered equal volumes of only vehicle.

Tissues examined in both genders included: Brain (four sections), including any four of the levels I through VII as recommended by the Society of Toxicologic Pathology (Bolon et al., 2013) and the National Toxicology Program (Rao et al., 2011 and Rao et al., 2014); lung, heart, kidney, adrenal gland, liver, and spleen. For the reproductive tissues, the left testis and epididymis from each male rat were stained with Periodic acid-Schiff-hematoxylin stain to elucidate acrosomal caps and basement membranes. Stage-aware evaluation of spermatogenesis was performed (Creasy, 1997). In females, samples from the ovary, uterus, and cervix with or without vagina were collected and evaluated in the context of what stage of the estrous cycle the animal was in (i.e., pro-estrus, estrus, metestrus or diestrus).

Statistical Analysis Methods of Histologic Evaluations

Analyses were conducted separately for male and female animals. Tissues from male rats from a Control group (n=9) and High Dose group (n=10) were evaluated microscopically, and the scores for 25 histologic changes ('metrics') were stratified across seven tissue types (organs). Reproductive tissues (testis, epididymis) from an additional 10 male rats in a medium (830 mg/kg) dose group were evaluated, and 10 histologic changes (metrics) identified, affecting at least one animal. Thirty-three histologic changes (metrics) were identified in tissues from female rats from a Control group (n=10) and High Dose group (n=10). Each animal was scored or 'classified' per metric on a 0-4 classification scale, where 0=NO Lesion, 1=change affecting <10% of section, 2=<20%, 3=<50%, 4=>50% of tissue affected. All individual animal scores are present as **Appendix B**.

Due to overall small sample sizes, a Fisher's Exact Test was used to compare the distribution of animals classified on the 0-4 scale for the two respective Control and High Dose groups. A p-value < .05 indicates a statistically significant result, meaning the distribution was different between the Control and High Dose groups. SAS® 9.4 was used to analyze the data. The p-values and incidence tables are present as **Appendix C**.

3. ANIMALS

The nine male Sprague-Dawley control rats were: 14-0635, -0639, -0640, -0657, -0658, -0673, -0674, -687, -0688. The 10 male high-dose rats were: 14-0627, -0628, -0631, -0632, -0637, -0638, -0651, -0652, -0671, -0672. One of the control rats had no olfactory lobe tissue present for evaluation. Three of the high-dose rats had no olfactory lobe tissue. A 10th rat (14-0636) was euthanized on Day 6. Tissues were taken and evaluated from this rat but, to be consistent with the other animals, the results are not included in the group results. Upon identification of lesions in the reproductive tissue of high-dose male rats, testis, and epididymis of male rats administered a 'medium' dose of MeNQ were also examined (14-0655, -0656, -0661, -0662, -0669, -0670, -0679, -0680, -0685, and -0686).

The 10 female Sprague-Dawley control rats were: 14-0694, -0695, -0700, -0701, -0716, -0717, -0730, --0731, -0732, and -0733. The 10 high-dose females were: -0696, -0697, -0706, -0707, -0724, -0725, -0734, -0735, -0738, -0739. All were approximately 77 days old at necropsy.

4. OVERALL CONCLUSION

There were no significant (statistically or biologically) effects in exposed rats at these doses. Tables 1 and 2 list incidence of histologic changes in males and females, respectively. There were no histopathologic changes in high-dose rats that were not comparably present in control rats. Those histologic changes that were present were either also observed in control rats or are known, published background lesions (McInnes, 2012). Therefore, MeNQ does not appear to be toxic at the doses tested in subacute oral administration in Sprague-Dawley rats.

5. COMBINED RESULTS WITH DISCUSSION

5.1 Lung

Seven of 10 control male rats and 8 of 10 high-dose male rats had scattered hemorrhage affecting less than 10% of the evaluated pulmonary tissue (called 'minimal'). Listed in Table 1 are female pulmonary lesions. They are comparable to males in distribution across controls and high-dose animals with the exception of alveolar hemorrhage. Although only 3 of 10 control females had minimal alveolar hemorrhage, 8 of 10 high-dose females did. Two of those eight high-dose females had 'mild' hemorrhage, which suggests a dose-response; however, the opinion of this pathologist is that the vast majority of this hemorrhage is perimortem and unrelated to the administration of MeNQ. Ante-mortem reaction to the hemorrhage was observed in only 2 of the 26 affected animals and that was minimal at worst. Ante-mortem hemorrhage may have been present in -0639 (Control) and -0627 (high dose) as evidenced by the presence of a few macrophages with engulfed erythrocytes. Perimortem pulmonary hemorrhage is a common observation with any kind of euthanasia in small rodents, especially secondary to terminal cardiac phlebotomy. Given the presence of resident alveolar macrophages, it may be anticipated that a few of them would begin engulfing erythrocytes in an animal inadvertently injured during terminal phlebotomy. Animals were randomized prior to euthanasia, so it is unlikely that the specific euthanasia technique is involved. Terminal phlebotomy is the most likely source of perimortem alveolar hemorrhage. One would expect that randomization would prevent a dose-response, if the same phlebotomist used the exact same approach for the terminal blood draw. A change in phlebotomist (not present in this case), or merely a change in hand position or angle of hypodermic needle can affect the amount of perimortem alveolar hemorrhage, as can inconsistent lung lobe trimming prior to fixation. The cause of the higher incidence of alveolar hemorrhage in high-dose females is unclear, but there are no indications of it occurring antemortem. Coagulation profile data were not available for examination. In the opinion of this pathologist, the alveolar hemorrhage is unrelated to an effect of the test article but a treatment effect cannot be ruled in or out definitively at this time. Statistically, there was neither a dose response, nor a statistically significant response.

One incident of pulmonary osseous metaplasia (14-0725/high dose) and one of eosinophilic infiltrate (-0706/high dose) are known background lesions. One high dose male rat (-0671) had focal neutrophils with a few macrophages, which indicates a minimal focus of inflammation. Other single incidents of background lesions are not discussed.

5.2 Thymus

A normal thymus was observed in all 20 male rats. Minimal perimortem hemorrhage is expected and was observed in females. Female rats were more likely to exhibit thymic epithelial remnants or lesions associated with such remnants (0738/high dose).

5.3 Heart

Two male control rats exhibited minimal focal myocardial hemorrhage (perimortem) and two others had focal leukocytic infiltrates. The female hearts were all normal.

5.4 Kidney

Many male and fewer female rats of both high dose and control groups exhibited pyknotic nuclei of the tubules of the inner stripe of the outer medulla (ISOM). The ISOM is composed of thin descending tubules, thick ascending tubules and outer medullary collecting ducts. The pyknotic nuclei, which are often associated with hyper-eosinophilic, contracted cytoplasm resemble early apoptosis and are a reflection of those cells' extreme sensitivity to hypoxia that occurs immediately post-mortem. In this study, it appears to be strictly a background finding.

Approximately half of each group of male rats and virtually all of the females (controls and high-dose) had minimal protein in many tubules. The pallor of the fluid suggests it could be glomerular filtrate rather than pure protein. This was determined to be within normal limits for this age and strain of rat based on comparison with controls. A few rats of each gender, controls and MeNQ-exposed, exhibited rare basophilic cortical tubules and/or had minimal interstitial lymphocytic infiltrates. Rats as young as 2 months of age have been reported to exhibit early, minimal changes associated with the development of chronic progressive nephropathy (CPN) and progress throughout the life of the rat.

5.5 Adrenal gland

One male control rat had ectopic medullary cells may be present in the zona glomerulosa. This is a known background change. Among the female rats, only the high-dose group exhibited minimal to mild amounts of extra-capsular cortical cells (resembling zona fasciculata). This is generally considered a background change but could be of interest if glucocorticoids were concurrently elevated and/or adrenal gland weights were increased (i.e., if corroborated by additional data). The zona fasciculate cells were histologically normal in appearance.

5.6 Liver

There were no significant changes in the liver in the examined rats. Minimal lymphocytic portal or centrilobular infiltrates were common in both sexes. One small portal focus of extramedullary hematopoiesis was observed in one control male. It is uncommon in adult rats. Micro-foci of lymphocytic, lymphohistiocytic or histiocytic infiltrates were occasionally seen surrounding bile ductules of portal triads, adjacent to central veins, or randomly scattered in the parenchyma of both sexes. At the amounts observed in these rats, these are background lesions.

5.7 Spleen

No significant histologic changes were observed. Table 1 lists all tissues with histologic changes in control or treated female rats.

Table 1. Female Rat Histologic Findings: High Dose MeNQ Exposure versus Controls

	Vehicle Control (0 mg/kg/day of MeNQ)					Total	High dose (1250 mg/kg/day)					
	None	Minimal	Mild	Moderate	Marked		None	Minimal	Mild	Moderate	Marked	Total
Lung												
Osseous metaplasia	10	0	0	0	0	10	9	1	0	0	0	10
Infiltrate, eosinophilic	10	0	0	0	0	10	9	1	0	0	0	10
Bronchial cartilage mineralization	9	1	0	0	0	10	9	1	0	0	0	10
Erythrophagocytosis	9	1	0	0	0	10	8	2	0	0	0	10
Fibrin, intraalveolar	8	2	0	0	0	10	8	2	0	0	0	10
Crystals, intra-alveolar or septal	10	0	0	0	0	10	9	1	0	0	0	10
Infiltrate, alveolar, histiocytic	8	2	0	0	0	10	7	3	0	0	0	10
Alveolar hemorrhage	7	3	0	0	0	10	2	6	2	0	0	10
Thymus						10						10
Infiltrate, eosinophilic	9	1	0	0	0	10	10	0	0	0	0	10
Epithelial tube remnants, cysts	7	2	1	0	0	10	4	2	3	0	1	10
Hemorrhage	8	2	0	0	0	10	8	1	1	0	0	10
Heart												
Fibrosis	9	1	0	0	0	10	10	0	0	0	0	10
Hemorrhage, myocardial	10	0	0	0	0	10	9	1	0	0	0	10
Infiltrate, leukocytic	10	0	0	0	0	10	9	1	0	0	0	10
Pericardial fat, lymphocytic infiltrate	10	0	0	0	0	10	9	1	0	0	0	10
Infiltrate, lymphocytic	10	0	0	0	0	10	9	1	0	0	0	10
Kidney												
Congestion	9	1	0	0	0	10	10	0	0	0	0	10
Infarct	9	1	0	0	0	10	10	0	0	0	0	10
Mineral	9	1	0	0	0	10	9	1	0	0	0	10
Tubule basophilia	7	3	0	0	0	10	9	1	0	0	0	10
Tubule protein	0	9	1	0	0	10	2	7	1	0	0	10
Infiltrate, lymphoplasmacytic, interstitia	6	4	0	0	0	10	8	2	0	0	0	10
Adrenal Gland												
Ectopic medullary cells	10	0	0	0	0	10	8	2	0	0	0	10
Z. glomerulosa hyperplasia	10	0	0	0	0	10	9	1	0	0	0	10
Extracapsular cortical cells	10	0	0	0	0	10	6	2	2	0	0	10
Liver												
Infiltrate, inflammatory, centrilobular	5	4	0	0	0	9	9	1	0	0	0	10
Vacuoles, portal-centric	6	2	1	0	0	9	9	1	0	0	0	10
Hepatocellular single-cell necrosis	6	3	0	0	0	9	8	2	0	0	0	10
Infiltrate, portal, inflammatory	6	3	0	0	0	9	5	5	0	0	0	10
Infiltrate, random	5	4	0	0	0	9	8	2	0	0	0	10
Ovary												
Sertoliform tubules	10	0	0	0	0	10	9	1	0	0	0	10
Ectatic lymphatics	0	8	2	0	0	10	0	5	2	3	0	10

Histologic alterations (lesions) were scored: 1 (min) <5% (1 of 20) tubules affected; 2 (mild) 6-20% (2-4 of 20) tubules; 3 (moderate) 21-50% (5-10 of 20) tubules; 4 (marked) 51-75% (11-15 of 20) tubules.

5.8 Male Reproductive Tissue Evaluation

The entire section was initially screened at 4–10X. If one of the below lesions was spotted, then the following criteria were used to score changes in the testis and epididymis: 1 (minimum)= <5% (1 of 20) tubules affected; 2 (mild)= 6–20% (2–4 of 20) tubules; 3 (moderate)= 21–50% (5–10 of 20 tubules); 4 (marked)= 51–75% (11–15 of 20 tubules); 5 (severe)= >75% (>15 of 20) tubules.

The overall histologic size (a crude gauge of 'normality') is that the adult rat testis diameter is usually about the width of a 2X-power field as seen through the eyepiece. Only one control rat and one high-dose rat had a testicular diameter smaller than a 2X field. Table 2 lists all tissues with lesions in control or treated male rats.

Table 2. Male Rat Histologic Findings: High Dose MeNQ Exposure versus Controls

	Vehicle Control (corn oil)						High dose (1250mg/kg/day)					
	None	Minimal	Mild	Moderate	Marked	Total	None	Minimal	Mild	Moderate	Marked	Total
Lung												
Infiltrate,granulocytic, alveolar	9	0	0	0	0	9	9	1	0	0	0	10
Infiltrate, alveolar, histiocytic	9	0	0	0	0	9	9	1	0	0	0	10
Infiltrate, interstitial,histiocytic	8	1	0	0	0	9	10	0	0	0	0	10
Alveolar hemorrhage	3	6	0	0	0	9	2	8	0	0	0	10
Thymus												
Hemorrhage	8	1	0	0	0	9	9	1	0	0	0	10
Heart												
Hemorrhage, myocardial	7	2	0	0	0	9	10	0	0	0	0	10
Infiltrate, leukocytic	8	0	1	0	0	9	10	0	0	0	0	10
Edema, perivascular	9	0	0	0	0	9	9	1	0	0	0	10
Infiltrate, lymphocytic	9	0	0	0	0	9	9	1	0	0	0	10
Infiltrate, histiocytic	7	2	0	0	0	9	10	0	0	0	0	10
Kidney												
Atrophy,fibrosis, glomerular	9	0	0	0	0	9	9	1	0	0	0	10
Pyknosis in inner stripe collecting ducts	2	6	1	0	0	9	2	4	4	0	0	10
Tubule basophilia	6	3	0	0	0	9	8	2	0	0	0	10
Tubule protein	5	4	0	0	0	9	6	4	0	0	0	10
Infiltrate, lymphoplasmacytic, interstitial	5	4	0	0	0	9	6	4	0	0	0	10
Adrenal Gland												
Ectopic medullary cells	7	1	0	0	0	8	10	0	0	0	0	10
Liver												
Infiltrate, mononuclear, centrilobular	7	2	0	0	0	9	6	4	0	0	0	10
Hepatocellular single-cell necrosis	8	1	0	0	0	9	9	1	0	0	0	10
Infiltrate, histiocytic, random	4	5	0	0	0	9	7	3	0	0	0	10
Infiltrate, neutrophilic, focal	8	1	0	0	0	9	10	0	0	0	0	10
Congestion	6	3	0	0	0	9	9	1	0	0	0	10
Infiltrate, portal, lymphocytic	2	7	0	0	0	9	6	4	0	0	0	10
Spleen												
Germinal Centers, increased	7	2	0	0	0	9	10	0	0	0	0	10
Testis												
Retained spermatids	9	0	0	0	0	9	9	1	0	0	0	10
Sertoli-only tubules	9	0	0	0	0	9	9	1	0	0	0	10
Multinucleate giant cells	9	0	0	0	0	9	9	1	0	0	0	10
Sloughed germ cells in lumen	9	0	0	0	0	9	9	1	0	0	0	10
Gaps in germ cells production	9	0	0	0	0	9	9	0	1	0	0	10
Sertoli cell Vacuoles	8	1	0	0	0	9	7	1	2	0	0	10
Apoptotic cells	9	0	0	0	0	9	9	1	0	0	0	10
Epididymus												
Leukocyte infiltration	7	2	0	0	0	9	6	4	0	0	0	10
Ectatic lymphatics with proteinaceous fluid	9	0	0	0	0	9	7	3	0	0	0	10
Dilatation	9	0	0	0	0	9	6	2	2	0	0	10

Histologic alterations (lesions) were scored: 1 (min) <5%(1 of 20) tubules affected; 2 (mild) 6-20% (2-4 of 20) tubules; 3 (moderate) 21-50% (5-10 of 20) tubules; 4 (marked) 51-75% (11-15 of 20) tubules.

There was a mild increase in Sertoli cell vacuoles, with rare gaps in germ cell production, retention of a few spermatids in high-dose males over control males, but these were not statistically significant. The Sertoli cell vacuoles were mild in two (0628 and 0637) and minimal in one (0631) of the 10 high-dose rats. Sertoli cell vacuoles were minimal to mild in five medium-dose animals and in a few controls (Appendix B). A sixth medium-dose animal (0661) had extensive testicular lesions, which may have arisen prior to exposure to MeNQ. This animal is believed to be an outlier. The largest and most numerous vacuoles were in the high-dose group. Two high-dose rats up to 20% of tubule profiles in the section had at least one vacuole, which contrasts with the presence of vacuoles in 1/10 control rats, with fewer than 5% of the tubules affected in that rat.

Two of the 10 high-dose group animals had 2–3 Sertoli-only seminiferous tubules. This means germ cells were virtually absent from those tubules. Such tubules are often much smaller than normal ones. Sertoli-only tubules can be part of normal anatomy when located near the tubuli recti at the periphery of the

section, which is most likely the case in rat 0631. When not part of the tubuli recti, their presence in low numbers can be a background lesion. "Up to five Sertoli-only tubules" reportedly is within normal limits (McInnes, 2012). Comparison with controls, however, is essential to determine whether the presence and number of Sertoli-only tubules are pathological in a particular study. Since control rats had No Sertoli-only tubules, their presence in high-dose rat 0632 was noted.

There were no observed changes in Sertoli cell size. Leydig cells appeared normal in size, shape and number. The only luminal debris was most likely residual bodies after maturing spermatids detach their extraneous cytoplasm (usually apparent during stage VIII and IX). The Sertoli cells phagocytize residual bodies and translocate them to the basal side of the cell where they are 'digested' and the components recycled. Virtually all germ cells appeared within normal limits; multinucleate cells were found only in one examined testis (0628, high-dose) as was the case with single necrotic germ cells.

5.9 Epididymis

The initial segment, caput, corpus, and cauda were examined, as were the following cell types: Main, Basal, Apical, Clear, intraepithelial lymphocytes and macrophages. Two of nine control rats and 4 of 10 high-dose rats had minimal leukocytic infiltrates in the epididymal interstitium. Three of ten high-dose animals and 1 of 10 control rat exhibited expanded caudal epididymis luminal profiles which generally represents filling with mature sperm. Four of ten high-dose rats exhibited protein in mildly dilated lymphatic vessels in contrast to none in the control group. However, none of these findings convincingly suggests a toxic effect of the test article.

5.10 Female Reproductive Tissue Evaluation

Female Reproductive Tissue Evaluation: Histologic examination of ovary, uterus, cervix, and vagina indicated that these female rats were in all four stages of the estrus cycle. No single stage predominated in either group (Table 3). These females lacked two features commonly observed in cycling female rats (edematous uterine tissues and pigment-laden macrophages), probably related to their young age. Ovary and uterus of control tissues were more often evaluated to be in diestrus than high-dose rats (5 of 5 rats compared to 0 of 5 rats, p= 0.033). The reason for this is unclear but not likely to be biologically significant given that the reproductive cycle in female rats is 4-5 days long, approximately one-third of the length of the study, and controls and high-dose females were comparably observed to be in proestrus (4 controls versus 5 high-dose rats) or estrus (1 control versus 2 high-dose rats). This is an example of a p-value raising undue attention on a finding.

Pathology was not observed in female reproductive tissues of these rats.

Table 3. Reproductive Cycle Histologic Observations of Control and High Dose 77-day old Female Rats

	Controls	High-dose
Proestrus	3/10	5/10
Estrus	1/10	2/10
Diestrus	4/10	0/10
Metestrus	0/10	3/10
Out-of-Sync*	2/10	0/10
*=for 14-0695 and 14-0701 control female rats, the vagina was lined with abundant sloughing keratinized squamous epithelium (estrus) but the uterus of 0695 appears to be in proestrus (luminal dilation, thickened columnar epith, no degeneration) and the uterus of 0701 has mitotic figures, tall columnar epith, rare apoptotic cells, NO luminal dilation, consistent with early proestrus or late diestrus.		

6. STORAGE OF STUDY MATERIALS AND RECORDS RETENTION

The study records and pathology final report will be archived and maintained at or under the direction of the Army Public Health Center (Provisional) (APHC (Prov))'s Toxicology Portfolio (TOX), according to TOX SOPs and U.S. Environmental Protection Agency (EPA) requirements. The Pathology specimens will also be archived and maintained at or under the direction of APHC (Prov) Toxicology Portfolio, according to TOX SOP and EPA requirements.

7. PHOTOMICROGRAPHS

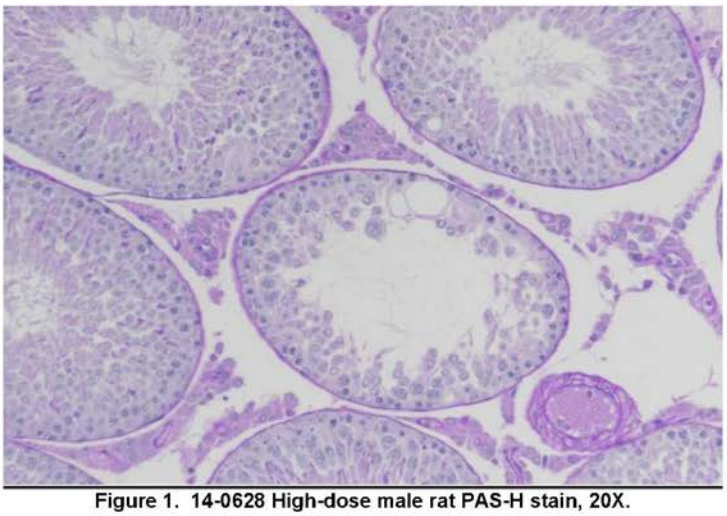

Figure 1. 14-0628 High-dose male rat PAS-H stain, 20X.

Sertoli cell vacuoles with absence of elongating spermatids are evident in the central tubule, which is at approximately Stage IV-VII and, therefore, should have elongated spermatids. A gap in germ cells is evident in the tubule above and to the left.

Figure 2. 14-0658 PAS-H Control rat (40X). This is a typical Stage XIV tubule.

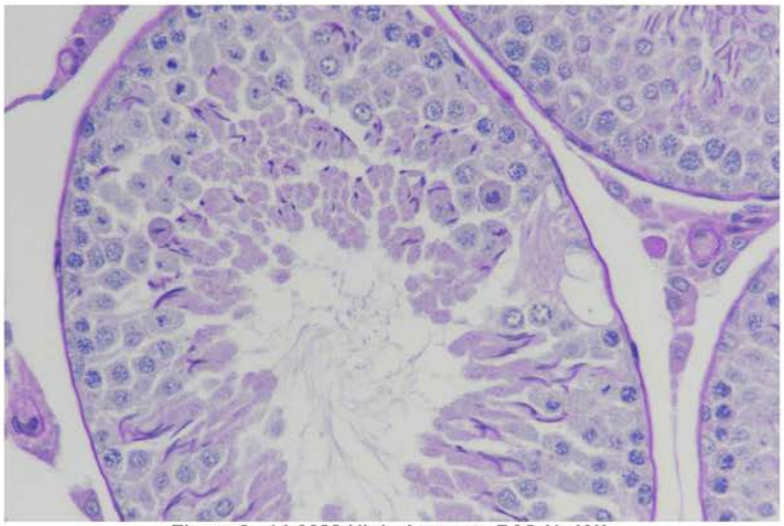

Figure 3. 14-0628 High-dose rat. PAS-H. 40X.

Same rat illustrating vacuoles in Sertoli cells and an associated gap in germ cell progression. This is a Stage XIV tubule with one evident abnormal mitotic figure.

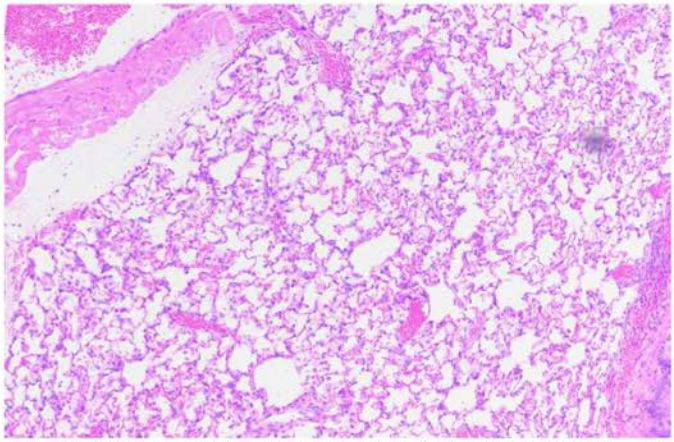

Figure 4. 14-0738 Female High-dose rat. H&E. 10X Mild alveolar hemorrhage.

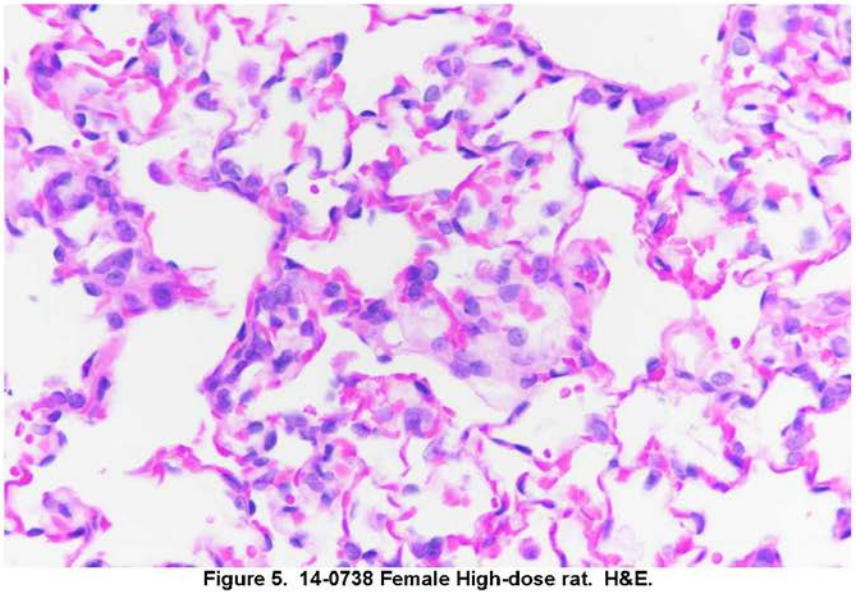

Figure 5. 14-0738 Female High-dose rat. H&E.

40X Mild alveolar hemorrhage with fibrin and increased numbers of alveolar macrophages, suggesting antemortem process.

APPENDIX A

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APPENDIX B

INDIVIDUAL ANIMAL OBSERVATIONS/SCORES

MeNQ High-Dose Male Rat Histological Findings:

30-14-07-01 Methyl Nitroguanidine High-dose Male Rat Histological Findings

TISSUE/Lesion Animal #:	14-0635	14-0636	14-0639	14-0640	14-0657	14-0658	14-0673	14-0674	14-0687	14-0688	14-0627	14-0628	14-0631	14-0632	14-0637	14-0638	14-0651	14-0652	14-0672	14-0671
77 d-old Male Sprague-Dawley Rats	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	High	High	High	High	High	High	High	High	High	High
Anterior brain and hippocampus	P*	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Cerebellum, brainstem (level 4,6,7)	P	P	NP*	NP	NP	P	NP	P	P	P	P	P	P	P	P	P	P	P	P	P
Hippocampus- Br -4.36mm	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Lung																				
Infiltrate, granulocytic, alveolar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
infiltrate, alveolar, histiocytic	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Infiltrate, interstitial, histiocytic	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage, alveolar	0	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1
Thymus																				
Hemorrhage	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Lymph node (not required)		P																		
Lymph node-germinal centers	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart																				
Hemorrhage, myocardial	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltrate, leukocytic	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Edema, perivascular	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Infiltrate, lymphocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Infiltrate, histiocytic	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney																				
Atrophy,fibrosis, glomerular	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Protein tubules	0	1	1	0	0	0	1	1	1	0	1	1	0	0	0	1	1	0	0	0
Pyknosis in inner stripe collecting	1	3	0	2	1	0	1	1	1	1	2	1	2	0	1	2	2	1	0	1
Basophilia, cortical tubules	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0
infiltrate, lymphoplasmacytic,	0	0	1	1	0	0	1	0	0	1	0	0	0	1	1	1	0	1	0	0
Adrenal glands					NP															
Ectopic medullary cells	0	0	0	0		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver																				
Infiltrate, lymphocytic, centrilobular	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	0
Hepatocellular single-cell necrosis	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Infiltrate, portal, lymphocytic	1	0	1	0	1	1	0	1	1	1	0	0	0	0	1	0	1	1	1	0
Infiltrate, histiocytic, random	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	1	0	1	0
Infiltrate, neutrophilic, focal	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Congestion	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0
Hematopoiesis, extramedullary	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spleen																				
Germinal Centers, increased	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

14-0636 was euthanized early. Data not included in statistical analysis.

* P and NP signify the tissue was Present or Not Present, respectively. Any tissue with an alteration (lesion) was, instead, given a numerical score: 0=NO Lesion, 1=(mild) change affecting <10% of section, 2=Mod <20%, 3=<50%, 4=>50% of tissue affected.

Blue highlighting indicates those histologic changes that were noted in 1 or more male rats but not in females.

MeNQ High-, Medium-, and Vehicle-Dosed Male Rat Reproductive Tissue:

MeNQ High-, Medium- and Vehicle-dosed Male Rat Reproductive Tissue Histological Findings

Animal ID:	14-0635	14-0639	14-0640	14-0657	14-0658	14-0673	14-0674	14-0687	14-0688	14-0627	14-0628	14-0631	14-0632	14-0637	14-0638	14-0651	14-0652	14-0671	14-0672	14-0655	14-0656	14-0661	14-0662	14-0669	14-0670	14-0679	14-0680	14-0685	14-0686
Tissue/Lesion	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	High	High	High	High	High	High	High	High	High	High	med	med	med	med	med	med	med	med	med	med
TESTIS																													
Reduced testis diameter (norm=width of 2X field)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Reduction in elongating spermatids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Protein between seminiferous tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0
Retained spermatids (visible in Stage IX-X)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sertoli-only tubules	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Multinucleate giant cells	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Sloughed germ cells in lumen	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Gaps in germ cells production	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Sertoli cell Vacuoles	0	0	0	1	0	0	0	0	0	0	2	1	0	2	0	0	0	0	0	1	0	4	2	0	2	0	0	1	2
Apoptotic cells in other than stage VIII-IX	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0	0	0
EPIDIDYMIS																													
Spermatic granuloma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Leukocyte infiltration	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	0	0	3	0	0	0	0
Change in constitutive cells (e.g., clear cells) in cauda epith	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypospermia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	2	0	0	0	0
Abnormal cells in lumen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	1	0	0	0	0	0
Ectatic lymphatics with proteinaceous fluid	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Dilatation	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0

Any histologic alteration (lesion) was given a score: 1 (min) <5%(1 of 20) tubules affected; 2 (mild) 6-20% (2-4 of 20 tubules; 3 (moderate) 21-50% (5-10 of 20 tubules); 4 (marked) 51-75% (11-15 of 20 tubules; 5 (severe)

Lesion scores based on an average of the number of affected tubules per 20.

MeNQ-Exposed Adult Female Sprague-Dawley Rats: Control vs High Exposure:

MeNQ-Exposed Adult Female Sprague-Dawley Rats: Control versus High-Exposure Histologic Findings

TISSUE/Lesion Animal #:	14-0694	14-0695	14-0700	14-0701	14-0716	14-0717	14-0730	14-0731	14-0732	14-0733	14-0696	14-0697	14-0706	14-0707	14-0724	14-0725	14-0734	14-0735	14-0738	14-0739
Approximately 77 d-old Females	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	Ctrl	High	High	High	High	High	High	High	High	High	High
Brain Levels 1-3	P*	P	p	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Brain Levels 4-7	P	P	p	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Lung																				
Osseous metaplasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Infiltrate, alveolar, histiocytic	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1
Infiltrate, eosinophilic	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Bronchial cartilage mineralization	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Erythrophagocytosis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Fibrin, intraalveolar	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0
Crystals, intra-alveolar or septal	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Hemorrhage, alveolar	1	0	0	0	0	0	1	0	0	1	1	0	2	1	1	1	1	1	2	0
Thymus																				
Epithelial tube remnants or cysts	0	1	0	0	1	0	0	0	0	2	0	0	0	2	2	1	1	0	4	2
Infiltrate, eosinophilic	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	1	0	0	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	0
Heart																				
Fibrosis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage, myocardial	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Infiltrate, leukocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pericardial fat, lymphocytic infiltrate	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Infiltrate, lymphocytic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Kidney																				
Congestion	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Protein in tubules (pale eosinophilic)	1	1	1	1	1	2	1	1	1	1	1	0	1	1	1	1	0	1	1	2
Infarct	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Mineral	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Basophilia, cortical tubules	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0
Infiltrate, lymphoplasmacytic, interstitial	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0
Adrenal glands	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Extracapsular cortical cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	2
Z. glomerulosa hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Ectopic medullary cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Liver					NP*															
Infiltrate, inflammatory centrilobular	1	1	1	1		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Vacuoles, porto-centric	0	0	0	1		0	0	2	0	1	0	0	0	0	0	0	0	1	0	0
Hepatocellular single-cell necrosis	1	0	1	0		0	1	0	0	0	0	1	0	1	0	0	0	0	0	0
Infiltrate, portal, inflammatory	1	1	0	0		0	1	0	0	0	0	0	0	1	1	0	0	1	1	1
Infiltrate, random	1	0	1	0		0	1	1	0	0	0	1	0	0	0	0	0	0	1	0
Spleen	NP	NP	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
OVARY																				
Sertoliform tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Ectatic lymphatics	2	2	1	1	1	1	1	1	1	1	1	1	1	3	1	3	2	2	1	3
Proestrus		X			X	x		X						X	X	X		X		X
Estrus							X				X	X								
Metestrus													X				X		X	
Diestrus	X		X	X					X	X										
UTERUS																				
Proestrus		X			X	X		X						X	X	X		X		X
Estrus							X				X	X								
Metestrus													X				X		X	
Diestrus	X		X	X					X	X										
VAGINA/CERVIX																				
Proestrus					X	X		X						X	X	X		X		X
Estrus		X		X			X				X	X								
Metestrus													X				X		X	
Diestrus	X		X						X	X										
All organs-Proestrus					X	X		X						X	X	X		X		X
All organs-Diestrus	X		X						X	X										
All organs-Metestrus													X						X	
1 of three tissues Out-of-Sync		X		X																

* P and NP signify the tissue was present or Not Present, respectively. Any tissue alteration (lesion) was given a numerical score: 0=NO Lesion, 1= minimal change, affecting <10% of section, 2= mild, <20%, 3= moderate, <50%, 4= marked, >50% of tissue affected.

Pink highlight indicates those histologic changes that were observed in one or more female rats but not in male rats.

APPENDIX C

STATISTICAL ANALYSIS OF HISTOLOGIC FINDINGS

The below screening test was performed first, simply comparing incidence of lesion 'present' or 'absent.' Subsequent analyses compared severity scores per lesion per group.

Incidence Comparisons (males somatic)

77 d-old Male Sprague-Dawley Rats	Control	High	Control Group vs High Dose Fisher's Exact P-value / Conclusion
Lung			
Infiltrate, granulocytic, alveolar	0/9	1/10	1.000 / No significant difference
infiltrate, alveolar, histiocytic	0/9	1/10	1.000 / No significant difference
Infiltrate, interstitial, histiocytic	1/9	0/10	1.000 / No significant difference
Hemorrhage, alveolar	6/9	8/10	0.628 / No significant difference
Thymus			
Hemorrhage	1/9	1/10	1.000 / No significant difference
Lymph node (not required)			
Lymph node-germinal centers	0/9	0/10	1.000 / No significant difference
Heart			
Hemorrhage, myocardial	2/9	0/10	0.211 / No significant difference
Infiltrate, leukocytic	1/9	0/10	1.000 / No significant difference
Edema, perivascular	0/9	1/10	1.000 / No significant difference
Infiltrate, lymphocytic	0/9	1/10	1.000 / No significant difference
Infiltrate, histiocytic	2/9	0/10	0.211 / No significant difference
Kidney			
Atrophy,fibrosis, glomerular	0/9	1/10	1.000 / No significant difference
Protein in tubules	4/9	4/10	1.000 / No significant difference
Pyknosis in inner stripe collecting ducts	7/9	8/10	1.000 / No significant difference
Basophilia, cortical tubules	3/9	2/10	0.628 / No significant difference
infiltrate, lymphoplasmacytic, interstitial	4/9	4/10	1.000 / No significant difference
Adrenal glands			
Ectopic medullary cells	1/9	0/10	1.000 / No significant difference
Liver			
Infiltrate, lymphocytic, centrilobular	2/9	4/10	0.628 / No significant difference
Hepatocellular single-cell necrosis	1/9	1/10	1.000 / No significant difference
Infiltrate, portal, lymphocytic	7/9	4/10	0.170 / No significant difference
Infiltrate, histiocytic, random	5/9	3/10	0.370 / No significant difference
Infiltrate, neutrophilic, focal	1/9	0/10	1.000 / No significant difference
Congestion	3/9	1/10	0.303 / No significant difference
Hematopoiesis, extramedullary	0/9	0/10	1.000 / No significant difference
Spleen			
Germinal Centers, increased	2/9	0/10	0.211 / No significant difference

Incidence Comparisons (males repro)					
Tissue	Control	Medium	High	Control Group vs Medium Dose Fisher's Exact P-value / Conclusion	Control Group vs High Dose Fisher's Exact P-value / Conclusion
TESTIS					
Reduced testis diameter (norm=width of 2X field)	0/9	1/10	0/10	1.000 / No significant difference	1.000 / No significant difference
Reduction in elongating spermatids	0/9	1/10	0/10	1.000 / No significant difference	1.000 / No significant difference
Protein between seminiferous tubules	0/9	2/10	0/10	0.474 / No significant difference	1.000 / No significant difference
Retained spermatids (visible in Stage IX-X)	0/9	0/10	1/10	1.000 / No significant difference	1.000 / No significant difference
Sertoli-only tubules	0/9	1/10	1/10	1.000 / No significant difference	1.000 / No significant difference
Multinucleate giant cells	0/9	1/10	1/10	1.000 / No significant difference	1.000 / No significant difference
Sloughed germ cells in lumen	0/9	1/10	1/10	1.000 / No significant difference	1.000 / No significant difference
Gaps in germ cells production	0/9	1/10	1/10	1.000 / No significant difference	1.000 / No significant difference
Sertoli cell Vacuoles	1/9	6/10	3/10	0.057 / No significant difference	0.582 / No significant difference
Apoptotic cells in other than stage VIII- IX	0/9	3/10	1/10	0.211 / No significant difference	1.000 / No significant difference
EPIDIDYMIS					
Spermatic granuloma	0/9	1/10	0/10	1.000 / No significant difference	1.000 / No significant difference
Leukocyte infiltration	2/9	2/10	4/10	1.000 / No significant difference	0.628 / No significant difference
Change in constitutive cells (e.g., clear cells) in cauda epith	2/9	0/10	1/10	0.211 / No significant difference	0.582 / No significant difference
Hypospermia	0/9	2/10	0/10	0.474 / No significant difference	1.000 / No significant difference
Abnormal cells in lumen	0/9	2/10	0/10	0.474 / No significant difference	1.000 / No significant difference
Ectatic lymphatics with proteinaceous fluid	0/9	0/10	3/10	1.000 / No significant difference	0.211 / No significant difference
Dilatation	0/9	0/10	4/10	1.000 / No significant difference	0.087 / No significant difference

MeNQ-Exposed Male Rats: High Dose versus Control Group:

Severity Level Comparisons (males Repro)		
Tissue	Fisher's Exact P-value	Conclusion
TESTIS		
Reduced testis diameter (norm=width of 2X field)	1.000	No significant difference
Reduction in elongating spermatids	1.000	No significant difference
Protein between seminiferous tubules	1.000	No significant difference
Retained spermatids (visible in Stage IX-X)	1.000	No significant difference
Sertoli-only tubules	1.000	No significant difference
Multinucleate giant cells	1.000	No significant difference
Sloughed germ cells in lumen	1.000	No significant difference
Gaps in germ cells production	1.000	No significant difference
Sertoli cell Vacuoles	0.474 / 0.211	No significant difference
Apoptotic cells in other than stage VIII- IX	0.474	No significant difference
EPIDIDYMIS		
Spermatic granuloma	1.000	No significant difference
Leukocyte infiltration	0.628 / 0.303	No significant difference
Change in constitutive cells (e.g., clear cells) in cauda epith	1.000	No significant difference
Hypospermia	1.000	No significant difference
Abnormal cells in lumen	1.000	No significant difference
Ectatic lymphatics with proteinaceous fluid	0.211	No significant difference
Dilatation	0.474	No significant difference

The second p-value in the Fisher's Exact P-value column represents the results of control tissue compared to medium-dose male rats.

Male Rats Exposed to High Dose MeNQ versus Controls, Somatic Tissues:

MeNQ-Exposed Male Rats: High Dose compared to Controls

Lung	Infiltrate, granulocytic, alveolar							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	9	1	0				10
Lung	infiltrate, alveolar, histiocytic							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	9	1	0				10
Lung	Infiltrate, interstitial, histiocytic							
		0	1	2	3	4	5	Total
	Control	8	1	0				9
	High	10	0	0				10
Lung	Hemorrhage, alveolar							
		0	1	2	3	4	5	Total
	Control	3	6	0				9
	High	2	8	0				10
Thymus	Hemorrhage							
		0	1	2	3	4	5	Total
	Control	8	1	0				9
	High	9	1	0				10
Lymph node (not required)	Lymph node-germinal centers							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	10	0	0				10
Heart	Hemorrhage, myocardial							
		0	1	2	3	4	5	Total
	Control	7	2	0				9
	High	10	0	0				10
Heart	Infiltrate, leukocytic							
		0	1	2	3	4	5	Total
	Control	8	0	1				9
	High	10	0	0				10
Heart	Edema, perivascular							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	9	1	0				10
Heart	Infiltrate, lymphocytic							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	9	1	0				10

Heart	Infiltrate, histiocytic							
		0	1	2	3	4	5	Total
	Control	7	2	0				9
	High	10	0	0				10
Kidney	Atrophy,fibrosis, glomerular							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	9	1	0				10
Kidney	Protein in tubules							
		0	1	2	3	4	5	Total
	Control	5	4	0				9
	High	6	4	0				10
Kidney	Pyknosis in inner stripe collecting ducts							
		0	1	2	3	4	5	Total
	Control	2	6	1				9
	High	2	4	4				10
Kidney	Basophilia, cortical tubules							
		0	1	2	3	4	5	Total
	Control	6	3	0				9
	High	8	2	0				10
Kidney	infiltrate, lymphoplasmacytic, interstitial							
		0	1	2	3	4	5	Total
	Control	5	4	0				9
	High	6	4	0				10
Adrenal glands	Ectopic medullary cells							
		0	1	2	3	4	5	Total
	Control	7	1	0				8
	High	10	0	0				10
Liver	Infiltrate, lymphocytic, centrilobular							
		0	1	2	3	4	5	Total
	Control	7	2	0				9
	High	6	4	0				10
Liver	Hepatocellular single-cell necrosis							
		0	1	2	3	4	5	Total
	Control	8	1	0				9
	High	9	1	0				10
Liver	Infiltrate, portal, lymphocytic							
		0	1	2	3	4	5	Total
	Control	2	7	0				9
	High	6	4	0				10

Liver	Infiltrate, histiocytic, random							
		0	1	2	3	4	5	Total
	Control	4	5	0				9
	High	7	3	0				10
Liver	Infiltrate, neutrophilic, focal							
		0	1	2	3	4	5	Total
	Control	8	1	0				9
	High	10	0	0				10
Liver	Congestion							
		0	1	2	3	4	5	Total
	Control	6	3	0				9
	High	9	1	0				10
Liver	Hematopoiesis, extramedullary							
		0	1	2	3	4	5	Total
	Control	9	0	0				9
	High	10	0	0				10
Spleen	Germinal Centers, increased							
		0	1	2	3	4	5	Total
	Control	7	2	0				9
	High	10	0	0				10

Summary of Fisher's Exact Test Results by Lesion in High-dose Exposed Male Reproductive Tissue:

Tissue	Fisher's Exact P-value	Conclusion
TESTIS		
Reduced testis diameter (norm=width of 2X field)	1.000	No significant difference
Reduction in elongating spermatids	1.000	No significant difference
Protein between seminiferous tubules	1.000	No significant difference
Retained spermatids (visible in Stage IX-X)	1.000	No significant difference
Sertoli-only tubules	1.000	No significant difference
Multinucleate giant cells	1.000	No significant difference
Sloughed germ cells in lumen	1.000	No significant difference
Gaps in germ cells production	1.000	No significant difference
Sertoli cell Vacuoles[1]	0.474 / 0.211	No significant difference
Apoptotic cells in other than stage VIII- IX	0.474	No significant difference
EPIDIDYMIS		
Spermatic granuloma	1.000	No significant difference
Leukocyte infiltration	0.628 / 0.303	No significant difference
Change in constitutive cells (e.g., clear cells) in cauda epith	1.000	No significant difference
Hypospermia	1.000	No significant difference
Abnormal cells in lumen	1.000	No significant difference
Ectatic lymphatics with proteinaceous fluid	0.211	No significant difference
Dilatation	0.474	No significant difference
Fisher's Exact Test p-value < 0.05 was considered satstically significant.		
[1] The second p-value was when comparing control to medium-dose tissues.		

Incidence Table by Lesion in High-dose versus Control Male Reproductive Tissues:

TESTIS	Reduced testis diameter (norm=width of 2X field)							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9			1			10
	High	10						10
TESTIS	Reduction in elongating spermatids							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9				1		10
	High	10						10

TESTIS	Protein between seminiferous tubules							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	8	1		1			10
	High	10						10
TESTIS	Retained spermatids (visible in Stage IX-X)							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	10						10
	High	9	1					10
TESTIS	Sertoli-only tubules							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9				1		10
	High	9	1					10
TESTIS	Multinucleate giant cells							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9	1					10
	High	9	1					10
TESTIS	Sloughed germ cells in lumen							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9		1				10
	High	9	1					10
TESTIS	Gaps in germ cells production							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9				1		10
	High	9		1				10
TESTIS	Sertoli cell Vacuoles							
		0	1	2	3	4	5	Total
	Control	9	1					10
	Med	4	2	3		1		10
	High	7	1	2				10

TESTIS	Apoptotic cells in other than stage VIII- IX							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	7	2	1				10
	High	9	1					10
EPIDIDYMIS	Spermatic granuloma							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	9				1		10
	High	10						10
EPIDIDYMIS	Leukocyte infiltration							
		0	1	2	3	4	5	Total
	Control	8	2					10
	Med	8	1		1			10
	High	6	4					10
EPIDIDYMIS	Change in constitutive cells (e.g., clear cells) in cauda epith							
		0	1	2	3	4	5	Total
	Control	8	2					10
	Med	10						10
	High	9	1					10
EPIDIDYMIS	Hypospermia							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	8		1			1	10
	High	10						10
EPIDIDYMIS	Abnormal cells in lumen							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	8	1				1	10
	High	10						10
EPIDIDYMIS	Ectatic lymphatics with proteinaceous fluid							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	10						10
	High	7	3					10
EPIDIDYMIS	Dilatation							
		0	1	2	3	4	5	Total
	Control	10						10
	Med	10						10
	High	6	2	2				10

Incidence of Reproductive Tissue Lesions of Medium-dose Male Rat versus Controls:

TESTIS	Grossly small testis diameter (norm=width of 2X field)							
		0	1	2	3	4	5	Total
	Ctrl	9		.	0	.	.	9
	Medium	9		.	1	.	.	10
TESTIS	Reduction in elongating spermatids							
		0	1	2	3	4	5	Total
	Control	9	.	.	.	0	.	9
	Medium	9	.	.	.	1	.	10
TESTIS	Protein between seminiferous tubules							
		0	1	2	3	4	5	Total
	Control	9	0	.	0	.	.	9
	Medium	8	1	.	1	.	.	10
TESTIS	Sertoli-only tubules							
		0	1	2	3	4	5	Total
	Control	9	.	.	.	0	.	9
	Medium	9	.	.	.	1	.	10
EPIDIDYMIS	Leukocyte infiltration							
		0	1	2	3	4	5	Total
	Ctrl	7	2	.	0	.	.	9
	Medium	8	1	.	1	.	.	10
EPIDIDYMIS	Change in constitutive cells (e.g., clear cells) in cauda epith							
		0	1	2	3	4	5	Total
	Ctrl	7	2	.	.	.	.	9
	Medium	10	0	.	.	.	.	10
EPIDIDYMIS	Hypospermia							
		0	1	2	3	4	5	Total
	Ctrl	9	.	0	.	.	0	9
	Medium	8	.	1	.	.	1	10
EPIDIDYMIS	Abnormal cells in lumen							
		0	1	2	3	4	5	Total
	Ctrl	9	0	.	.	.	0	9
	Medium	8	1	.	.	.	1	10

TESTIS	Multinucleate giant cells							
		0	1	2	3	4	5	Total
	Control	9	0					9
	Medium	9	1					10
TESTIS	Sloughed germ cells in lumen							
		0	1	2	3	4	5	Total
	Ctrl	9	.	0	.	.	.	9
	Medium	9	.	1	.	.	.	10
TESTIS	Gaps in germ cells production							
		0	1	2	3	4	5	Total
	Ctrl	9	.	0		0	.	9
	Medium	9	.			1	.	10
TESTIS	Sertoli cell Vacuoles							
		0	1	2	3	4	5	Total
	Ctrl	8	1	0	.	0	.	9
	Medium	4	2	3	.	1	.	10
TESTIS	Apoptotic cells in other than stage VIII- IX							
		0	1	2	3	4	5	Total
	Ctrl	9	0	0	.	.	.	9
	Medium	7	2	1	.	.	.	10
EPIDIDYMIS	Spermatic granuloma							
		0	1	2	3	4	5	Total
	Ctrl	9	.	.	.	0	.	9
	Medium	9	.	.	.	1	.	10

Incidence Comparisons (females)			
MeNQ-Exposed Adult Female Sprague-Dawley Rats: Control versus High-Exposure Histologic Findings	Control	High	Control Group vs High Dose Fisher's Exact P-value / Conclusion
Lung			
Osseous metaplasia	0/10	1/10	1.000 / No significant difference
Infiltrate, alveolar, histiocytic	2/10	3/10	1.000 / No significant difference
Infiltrate, eosinophilic	0/10	1/10	1.000 / No significant difference
Bronchial cartilage mineralization	1/10	1/10	1.000 / No significant difference
Erythrophagocytosis	1/10	2/10	1.000 / No significant difference
Fibrin, intraalveolar	2/10	2/10	1.000 / No significant difference
Crystals, intra-alveolar or septal	0/10	1/10	1.000 / No significant difference
Hemorrhage, alveolar	3/10	8/10	0.070 / No significant difference
Thymus			
Epithelial tube remnants or cysts	3/10	6/10	0.370 / No significant difference
Infiltrate, eosinophilic	1/10	0/10	1.000 / No significant difference
Hemorrhage	2/10	2/10	1.000 / No significant difference
Heart			
Fibrosis	1/10	0/10	1.000 / No significant difference
Hemorrhage, myocardial	0/10	1/10	1.000 / No significant difference
Infiltrate, leukocytic	0/10	1/10	1.000 / No significant difference
Pericardial fat, lymphocytic infiltrate	0/10	1/10	1.000 / No significant difference
Infiltrate, lymphocytic	0/10	1/10	1.000 / No significant difference
Kidney			
Congestion	1/10	0/10	1.000 / No significant difference
Protein in tubules (pale eosinophilic)	10/10	8/10	0.474 / No significant difference
Infarct	1/10	0/10	1.000 / No significant difference
Mineral	1/10	1/10	1.000 / No significant difference
Basophilia, cortical tubules	3/10	1/10	0.582 / No significant difference
Infiltrate, lymphoplasmacytic, interstitial	4/10	2/10	0.628 / No significant difference
Adrenal glands			
Extracapsular cortical cells	0/10	4/10	0.087/ No significant difference
Z. glomerulosa hyperplasia	0/10	1/10	1.000 / No significant difference
Ectopic medullary cells	0/10	2/10	0.474 / No significant difference
Liver			
Infiltrate, inflammatory centrilobular	4/9	1/10	0.141 / No significant difference
Vacuoles, porto-centric	3/9	1/10	0.303 / No significant difference
Hepatocellular single-cell necrosis	3/9	2/10	0.628 / No significant difference
Infiltrate, portal, inflammatory	3/9	5/10	0.650 / No significant difference
Infiltrate, random	4/9	2/10	0.350 / No significant difference
OVARY			
Sertoliform tubules	0/10	1/10	1.000 / No significant difference
Ectatic lymphatics	10/10	10/10	1.000 / No significant difference

Severity Level Comparison (females)		
MeNQ-Exposed Adult Female Sprague-Dawley Rats: Control versus High-Exposure Histologic Findings	Fisher's Exact P-value	Conclusion
Lung		
Osseous metaplasia	1.000	No significant difference
Infiltrate, alveolar, histiocytic	1.000	No significant difference
Infiltrate, eosinophilic	1.000	No significant difference
Bronchial cartilage mineralization	1.000	No significant difference
Erythrophagocytosis	1.000	No significant difference
Fibrin, intraalveolar	1.000	No significant difference
Crystals, intra-alveolar or septal	1.000	No significant difference
Hemorrhage, alveolar	0.370 / 0.474	No significant difference
Thymus		
Epithelial tube remnants or cysts	0.582	No significant difference
Infiltrate, eosinophilic	1.000	No significant difference
Hemorrhage	1.000	No significant difference
Heart		
Fibrosis	1.000	No significant difference
Hemorrhage, myocardial	1.000	No significant difference
Infiltrate, leukocytic	1.000	No significant difference
Pericardial fat, lymphocytic infiltrate	1.000	No significant difference
Infiltrate, lymphocytic	1.000	No significant difference
Kidney		
Congestion	1.000	No significant difference
Protein in tubules (pale eosinophilic)	0.582	No significant difference
Infarct	1.000	No significant difference
Mineral	1.000	No significant difference
Basophilia, cortical tubules	0.582	No significant difference
Infiltrate, lymphoplasmacytic, interstitial	0.628	No significant difference
Adrenal glands		
Extracapsular cortical cells	0.474	No significant difference
Z. glomerulosa hyperplasia	1.000	No significant difference
Ectopic medullary cells	0.474	No significant difference
Liver		
Infiltrate, inflammatory centrilobular	0.141	No significant difference
Vacuoles, porto-centric	0.582	No significant difference
Hepatocellular single-cell necrosis	0.625	No significant difference
Infiltrate, portal, inflammatory	0.650	No significant difference
Infiltrate, random	0.350	No significant difference

OVARY		
Sertoliform tubules	1.000	No significant difference
Ectatic lymphatics	0.350	No significant difference
Proestrus	1.000	No significant difference
Estrus	1.000	No significant difference
Metestrus	0.211	No significant difference
Diestrus	0.033	Control > High dose
UTERUS		
Proestrus	1.000	No significant difference
Estrus	1.000	No significant difference
Metestrus	0.211	No significant difference
Diestrus	0.033	Control > High dose
VAGINA/CERVIX		
Proestrus	0.650	No significant difference
Estrus	1.000	No significant difference
Metestrus	0.211	No significant difference
Diestrus	0.087	No significant difference
All organs-Proestrus	0.650	No significant difference
All organs-Diestrus	0.087	No significant difference
All organs-Metestrus	0.474	No significant difference
1 of three tissues Out-of-Sync	0.474	No significant difference

Incidence of MeNQ-Exposed Adult Female Sprague-Dawley Rats Compared to Controls:

Lung	Osseous metaplasia							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Lung	Infiltrate, alveolar, histiocytic							
		0	1	2	3	4	5	Total
	Control	8	2	0				10
	High	7	3	0	0	0		10
Lung	Infiltrate, eosinophilic							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10

Lung	Bronchial cartilage mineralization							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	9	1	0	0	0		10
Lung	Erythrophagocytosis							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	8	2	0	0	0		10
Lung	Fibrin, intraalveolar							
		0	1	2	3	4	5	Total
	Control	8	2	0				10
	High	8	2	0	0	0		10
Lung	Crystals, intra-alveolar or septal							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Lung	Hemorrhage, alveolar							
		0	1	2	3	4	5	Total
	Control	7	3	0				10
	High	2	6	2	0	0		10
Thymus	Epithelial tube remnants or cysts							
		0	1	2	3	4	5	Total
	Control	7	2	1				10
	High	4	2	3	0	1		10
Thymus	Infiltrate, eosinophilic							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	10	0	0	0	0		10
Thymus	Hemorrhage							
		0	1	2	3	4	5	Total
	Control	8	2	0				10
	High	8	1	1	0	0		10
Heart	Fibrosis							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	10	0	0	0	0		10

Heart	Hemorrhage, myocardial							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Heart	Infiltrate, leukocytic							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Heart	Pericardial fat, lymphocytic infiltrate							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Heart	Infiltrate, lymphocytic							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Kidney	Congestion							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	10	0	0	0	0		10
Kidney	Protein in tubules (pale eosinophilic)							
		0	1	2	3	4	5	Total
	Control	0	9	1				10
	High	2	7	1	0	0		10
Kidney	Infarct							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	10	0	0	0	0		10
Kidney	Mineral							
		0	1	2	3	4	5	Total
	Control	9	1	0				10
	High	9	1	0	0	0		10
Kidney	Basophilia, cortical tubules							
		0	1	2	3	4	5	Total
	Control	7	3	0				10
	High	9	1	0	0	0		10

Kidney	Infiltrate, lymphoplasmacytic, interstitial							
		0	1	2	3	4	5	Total
	Control	6	4	0				10
	High	8	2	0	0	0		10
Adrenal glands	Extracapsular cortical cells							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	6	2	2	0	0		10
Adrenal glands	Z. glomerulosa hyperplasia							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
Adrenal glands	Ectopic medullary cells							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	8	2	0	0	0		10
Liver	Infiltrate, inflammatory centrilobular							
		0	1	2	3	4	5	Total
	Control	5	4	0				9
	High	9	1	0	0	0		10
Liver	Vacuoles, porto-centric							
		0	1	2	3	4	5	Total
	Control	6	2	1				9
	High	9	1	0	0	0		10
Liver	Hepatocellular single-cell necrosis							
		0	1	2	3	4	5	Total
	Control	6	3	0				9
	High	8	2	0	0	0		10
Liver	Infiltrate, portal, inflammatory							
		0	1	2	3	4	5	Total
	Control	6	3	0				9
	High	5	5	0	0	0		10
Liver	Infiltrate, random							
		0	1	2	3	4	5	Total
	Control	5	4	0				9
	High	8	2	0	0	0		10

OVARY	Sertoliform tubules							
		0	1	2	3	4	5	Total
	Control	10	0	0				10
	High	9	1	0	0	0		10
OVARY	Ectatic lymphatics							
		0	1	2	3	4	5	Total
	Control	0	8	2				10
	High	0	5	2	3	0		10

APPENDIX M
MICRONUCLEUS ASSAY REPORT

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Litron Laboratories

Genetox Analysis by Flow Cytometry

October 30, 2014

Dr. Emily N. Reinke
US Army Public Health Command
Army Institute of Public Health
5158 Blackhawk Road
Aberdeen Proving Ground, MD 21010-5403

Dear Dr. Reinke:

Enclosed is the μicroFlow® Rat Micronucleus Analysis Report for Study: 30-14-07-01 (Litron GLP-2014-30-14-07-01). A Table of Contents for this report is found on page 2.

Please call if you have any questions.

Sincerely,

Dorothea K. Torous

Dorothea K. Torous
Principal Investigator

www.LitronLabs.com *3500 Winton Place, Rochester, NY 14623 (USA)* *585.442.0930*

MicroFlow® Rat Micronucleus Analysis Phase Report

Study:	30-14-07-01
Litron assigned Good Laboratory Practice Number:	GLP-2014-30-14-07-01
Dates Samples Received:	September 3, 2014
Experimental Phase Start Date:	September 3, 2014
Experimental Phase End Date:	September 3, 2014
Date of Phase Report:	October 30, 2014
Study Phase Plan:	M56MF-2014
Principal Investigator (PI):	Dorothea K. Torous
Test Site:	Litron Laboratories 3500 Winton Place Suite 1B Rochester, New York 14623
Study Director:	Emily N. Reinke, Ph.D., Biologist
Test Facility:	US Army Public Health Command Army Institute of Public Health 5158 Blackhawk Road Aberdeen Proving Ground, MD 21010-5403

Table of Contents

1. List of Figures and Tables

2. Good Laboratory Practice Compliance Statement

This phase of the study was conducted in compliance with the Principles of Good Laboratory Practice (GLP) by the Organisation for Economic Co-operation and Development (OECD), C(97)186/FINAL.

The study phase data have been reviewed by the Principal Investigator, who certifies that the information contained in this report accurately reflects and is supported by the study phase raw data and represents an appropriate conclusion within the context of the study phase design and evaluation criteria. Methods relating to the receipt and flow cytometric analysis of blood samples and the data presentation specified in the test facility's protocol and the MicroFlow Study Phase Plan were followed.

Principal Investigator Dorothea K. Torous Date 10/30/2014

Dorothea K. Torous, B.S.

3. Other Scientists Involved in the Study

Not applicable.

4. Quality Assurance Statement

This study phase has been subjected to inspection and the report has been audited by the Quality Assurance (QA) Unit of Litron Laboratories in accordance with GLP regulations for non-clinical laboratory studies by the Principles of Good Laboratory Practice, OECD [C(97)186/FINAL]. The report describes the methods and procedures used in the study phase and the reported results accurately reflect the raw data of this study phase.

The following inspections were performed by Nikki E. Hall, B.S., RQAP-GLP:

Date	Phase Inspected	Date Reported to PI and Test Site Management	Date Reported to Test Facility SD, QA and Management*
September 3, 2014	Solution Preparation	September 3, 2014	October 30, 2014
September 3, 2014	Sample Receipt	September 3, 2014	October 30, 2014
September 3, 2014	Sample Washing	September 3, 2014	October 30, 2014
September 3, 2014	Flow Cytometer Calibration	September 3, 2014	October 30, 2014
September 3, 2014	Sample Analysis	September 3, 2014	October 30, 2014
September 4, 2014	Check SOPs	September 4, 2014	October 30, 2014
September 4, 2014	Check Training	September 4, 2014	October 30, 2014
September 4, 2014	Check Data Sheets for Completion	September 4, 2014	October 30, 2014
September 4, 2014	Raw Data versus Report	September 4, 2014	October 30, 2014
September 4, 2014	Draft Report, Number 1, Check	September 4, 2014	October 30, 2014
October 30, 2014	MicroFlow Report Check	October 30, 2014	October 30, 2014

Quality Assurance Auditor Nikki E. Hall Date 30 Oct 2014
Nikki E. Hall, B.S., RQAP-GLP

* indicates the date that the inspection reports were sent to the Test Facility.

5. Summary

On September 3, 2014 Litron Laboratories received 30 fixed rat peripheral blood samples from sponsor's study 30-14-07-01. Samples were processed immediately upon receipt for flow cytometric analysis. Flow cytometric analysis of up to 20,000 reticulocytes (RETs) was carried out for each sample and the frequency of high CD71-positive reticulocytes (% RET) and the frequency of micronucleated high CD71-positive reticulocytes (% MN-RET) were determined for all samples.

6. Objective

The MicroFlow® Analysis Study Phase Plan described procedures for analyzing test facility-prepared rat peripheral blood samples for the presence of micronuclei (MN) using the MicroFlow procedure. Micronuclei were analyzed in the CD71-positive RET population and provided an indication of genotoxicity. The frequency of RET (% RET) among total red blood cells (RBCs) was also measured to provide an indication of bone marrow toxicity.

7. Materials and Methods

7.1. Experimental Procedures (Performed by Test Facility)

The test facility was responsible for following the procedures detailed in the MicroFlow kit manual supplied to the test facility. The supplies were provided as a MicroFlow kit by the test site. No deviations from the manual were noted.

7.2. Blood Sample Receipt

On September 3, 2014, 30 fixed rat peripheral blood samples were received from the test facility. Upon receipt, samples were processed immediately for flow cytometric analysis.

7.3. Blood Sample Preparation

The fixed blood samples were thawed and then washed by adding each aliquot to tubes containing 5 ± 1 ml of cold Hank's Balanced Salt Solution (HBSS) containing 1% fetal bovine serum. Cells were isolated by centrifugation, and the cell pellets were stored at 2 °C to 10 °C or on ice until staining. After analysis of each stained sample, the remaining cell pellets were discarded on September 15, 2014.

7.4. Staining for Identification of Cell Populations

An aliquot (20 µl) of each washed blood sample was added to 80 µl of a solution containing RNase (to degrade RNA, 1 mg/ml), a fluorescently labeled (fluorescein isothiocyanate; FITC) antibody to the transferrin receptor to stain RETs (anti-CD71-FITC, 10 µl/ml), and a fluorescently labeled antibody (phycoerythrin; PE) to label platelets (anti-CD61-PE, 5 µl/ml) in a base of HBSS. The samples were incubated in the staining solution for 30 ± 10 minutes at 2 °C to 10 °C and 30 ± 10 minutes at room temperature. After incubation, the cells were kept at 2 °C to 10 °C until analysis. A propidium iodide (PI) solution (2 ml ± 0.5 ml) was added to each sample immediately before flow cytometric analysis to stain all DNA, including MN in the cells.

7.5. Flow Cytometer Calibration

Methanol-fixed blood from rats infected with *Plasmodium berghei* was used to configure the flow cytometer before analysis. Whereas MN are relatively rare and exhibit a heterogeneous DNA content, parasitized cells are prevalent and have a homogenous DNA content. These characteristics make them ideal for calibrating the flow cytometer for the MN scoring application.

7.6. Analysis of Blood Samples

Each blood sample was analyzed by high-speed flow cytometry using CellQuest software, version 5.2 (Becton Dickinson, San Jose, CA). The stained cells were moved at a high velocity past an argon laser set to provide 488 nm excitation. Photomultiplier tubes collected the fluorescence emitted by each cell. Using the previously described staining procedure, the PI-stained DNA of the MN emitted a red fluorescence, the anti-CD71-FITC antibody emitted a high green fluorescent signal, and platelets were excluded based on their anti-CD61-PE fluorescence. Upon successful analysis of the stained samples, each was discarded.

7.7. Number of Cells Analyzed

For test facility samples, 20,000 RETs (CD71+) were evaluated for the presence of MN, except for Sample 14-0659.P where only 19,999 RETs (CD71+) were analyzed.

7.8. Data Provided

The number of normochromatic erythrocytes (NCEs), MN-NCEs, RETs and MN-RETs are provided for each sample. The frequency of MN-RETs was calculated as an indication of genotoxic potential and the % RET was determined to provide an indication of bone marrow toxicity.

7.9. Statistical Analysis of the Data

No statistical analyses were performed on the data, other than the calculations indicated above. The test facility is responsible for the evaluation and interpretation of results.

8. Records Maintained

The original study phase plan and original MicroFlow report will be sent to the test facility at the completion of the study phase. Litron will maintain copies of the report, protocol, study phase plan and original study specific records for two years following completion of the study. After the retention period, Litron will contact the test facility and study-specific records will either be discarded or sent to a test facility-requested site. Electronic copies of some records will be stored off-site (ESL Federal Credit Union, Brighton Henrietta Branch, Rochester, NY) in addition to storage at Litron Laboratories.

9. References

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- Dertinger SD, Torous DK and Tometsko KR (August 2000 [filed January 1999]) U.S. Patent No. 6,100,038.
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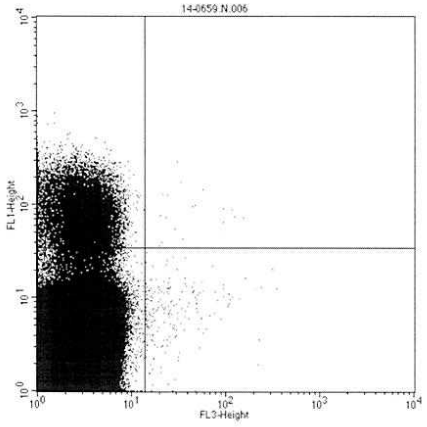
- Section 4 of the OECD Guidelines for the Testing of Chemicals: Mammalian Erythrocyte Micronucleus Test, Guideline 474 (Adopted 21st July 1997). This guideline states " . . . any appropriate mammalian species may be used provided it is a species in which the spleen does not remove micronucleated erythrocytes or a species which has shown an adequate sensitivity to detect agents that cause structural or numerical chromosome aberrations."
- Where applicable, GLP regulations for non-clinical laboratory studies as developed by the FDA (21 CFR 58). Please note that the computerized systems utilized for data acquisition, data analysis and report generation have undergone an internal validation guided by FDA GLP regulations. Litron is working towards 21 CFR part 11 compliance.
- Where applicable, ICH Harmonised Tripartite Guideline: Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, S2(R1), current Step 4 version dated 9 November 2011.
- Principles of GLP by OECD [C(97)186/FINAL].

Figures 1-2: Representative Bivariates

These representative bivariates of test facility submitted rat blood samples illustrate the resolution of the various erythrocyte populations. Lower Left quadrant = NCE [cells which are low in green and red fluorescence]; Lower Right = MN-NCE [cells high in red (PI) fluorescence]; Upper Left = high CD71 positive RET [cells with green (CD71) fluorescence]; Upper Right = high CD71 positive MN-RET [cells with both red and green fluorescence; the population of primary interest for this analysis].
Top Figure 1 – Sample Number 14-0659.N
Bottom Figure 2 – Sample Number 14.0660.P



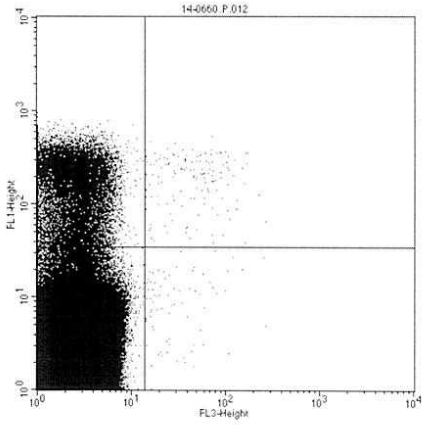


Table 1: Individual Animal Blood Data – Raw Data

Sample ID	Dose Group	No. NCE[1]	No. MN-NCE[2]	No. RET[3]	No. MN-RET[4]
14-0625	A	1033836	484	19965	35
14-0626	A	809476	315	19955	45
14-0627	D	746268	81	19980	20
14-0628	D	862327	457	19961	39
14-0633	A	1188703	537	19963	37
14-0634	A	1013797	196	19965	35
14-0635	C	1437487	195	19976	24
14-0637	D	740498	203	19947	53
14-0638	D	937939	230	19965	35
14-0639	C	799325	189	19979	21
14-0640	C	758419	168	19980	20
14-0655	B	601208	150	19969	31
14-0656	B	863124	126	19979	21
14-0661	B	908501	164	19979	21
14-0667	A	1060352	225	19980	20
14-0671	D	1532464	161	19981	19
14-0673	C	1173554	150	19978	22
14-0679	B	829016	277	19973	27
14-0680	B	608601	261	19960	40
14-0687	C	868388	293	19972	28
14-0647.N	Negative	1049078	505	19956	44
14-0648.N	Negative	794596	572	19959	41
14-0659.N	Negative	990596	199	19982	18
14-0660.N	Negative	532857	139	19969	31
14-0689.N	Negative	512050	149	19960	40
14-0647.P	Positive	2827987	709	19783	217
14-0648.P	Positive	3667935	328	19828	172
14-0659.P	Positive	2447533	383	19874	125
14-0660.P	Positive	946110	78	19855	145
14-0689.P	Positive	1075911	148	19784	216

[1] NCE = normochromatic erythrocytes
[2] MN-NCE = micronucleated normochromatic erythrocytes
[3] RET = young (high CD71-positive) reticulocytes
[4] MN-RET = young (high CD71-positive) micronucleated reticulocytes

Table 2: Individual Animal Blood Data – Calculated Data

Sample ID	Dose Group	%RET[a]	%MN-RET[b]
14-0625	A	1.90	0.18
14-0626	A	2.41	0.23
14-0627	D	2.61	0.10
14-0628	D	2.27	0.20
14-0633	A	1.65	0.19
14-0634	A	1.93	0.18
14-0635	C	1.37	0.12
14-0637	D	2.63	0.27
14-0638	D	2.09	0.18
14-0639	C	2.44	0.11
14-0640	C	2.57	0.10
14-0655	B	3.22	0.16
14-0656	B	2.26	0.11
14-0661	B	2.15	0.11
14-0667	A	1.85	0.10
14-0671	D	1.29	0.10
14-0673	C	1.68	0.11
14-0679	B	2.35	0.14
14-0680	B	3.18	0.20
14-0687	C	2.25	0.14
14-0647.N	Negative	1.87	0.22
14-0648.N	Negative	2.45	0.21
14-0659.N	Negative	1.98	0.09
14-0660.N	Negative	3.62	0.16
14-0689.N	Negative	3.76	0.20
14-0647.P	Positive	0.70	1.09
14-0648.P	Positive	0.54	0.86
14-0659.P	Positive	0.81	0.63
14-0660.P	Positive	2.07	0.73
14-0689.P	Positive	1.82	1.08

[a] % RET = frequency (%) of young (high CD71-positive) reticulocytes; calculated as [(Number of RET + Number of MN-RET)/ (Number of NCE + Number of MN-NCE + Number of RET + Number of MN-RET)] x 100

[b] % MN-RET = frequency (%) of young (high CD71-positive) micronucleated reticulocytes; calculated as [Number of MN-RET/(Number of RET + Number of MN-RET)] x 100;

Litron Laboratories

Rat Blood Study Phase Plan (M56MF-2014)
for MicroFlow[BASIC] Micronucleus Analysis Kit
An original signed document is required.

A. Contact Information

Test Facility Name and Address:

US Army Public Health Command
Army Institute of Public Health
5158 Blackhawk Rd
Aberdeen Proving Ground MD
21010-5403

Test Facility Study Director:

Name: Emily N Reinke
Phone: 410-436-2896
Fax:
Email: emily.n.reinke.civ@mail.mil

Test Site Name and Address:

Litron Laboratories
3500 Winton Place, Suite 1B
Rochester, New York 14623
phone: 585-442-0930
fax: 585-442-0934
info@litronlabs.com
www.litronlabs.com

Test Facility Lead QA Auditor:

Name: Mike Kefauver
Phone: 410-436-3752
Fax:
Email: michael.p.kefauver.civ@mail.mil

B. Study Information

Study ID: 30-14-07-01

GLP Number: GLP-2014-30-14-07-01 dlut 9/3/14
(if not provided, Litron will assign one)

[ERK] For analysis in compliance with GLP regulations, initial here. Provide the agency name that the data will be submitted to: self. Also, indicate which GLP regulations should be followed (FDA and/or OECD): OECD A copy of your protocol is required prior to sample analysis. For FDA GLP analyses, label samples with Sample ID, Study ID, Date Collected, Source (i.e., rat) and Type (i.e., blood). For OECD GLP, label samples with Unique ID and Sample ID.

[ ] For Non-GLP analysis, initial here if a final report is requested in addition to the electronic data file.

[ ] Initial here for statistical analysis of data. Additional charges apply. Contact Litron for details.

[ERK] All study phase specific records will be sent to your test facility after study phase completion, otherwise initial here to have records retained at Litron (see Section 8).

C. If applicable, please indicate any requested modifications to this Study Phase Plan:

D. Study Phase Plan Approval

Study Director Signature: Emily N Reinke **Date:** 2 Sept 2014

For Litron use only

Principal Investigator: D.K. Torous

Principal Investigator's Signature: Donna K. Torous Date: 9/5/2014

6. Data Provided

When possible, twenty thousand RET are analyzed per blood sample. In the event of bone marrow toxicity, the number analyzed may be reduced according to Litron's SOPs. The number of normochromatic erythrocytes (NCEs), MN-NCEs, RETs and MN-RETs are provided for each sample. The frequency of MN-RETs will be calculated as an indication of genotoxic potential. The % RET will be determined to provide an indication of bone marrow toxicity. Averages and standard deviations, per group and sex, will be provided (if known).

7. Evaluation and Interpretation of Results

No statistical analyses will be performed on the data, other than the calculations indicated above, and the test facility will be responsible for the evaluation and interpretation of results, unless the appropriate box in section B is initialed. If statistical analyses are requested, Litron's SOP for statistical evaluation will be followed.

8. Records Maintained

If requested in Section B, the original study phase plan, original MicroFlow report, and study-specific records (copies, if applicable) will be transferred to the test facility at the completion of the study phase. Litron will maintain copies of the report, protocol, and study phase plan, along with original study-specific records for two years following completion of the study. After the retention period, Litron will contact the sponsor and study-specific records will either be discarded or sent to the sponsor-requested facility. Electronic copies of some records will be stored off-site (ESL Federal Credit Union, Brighton Henrietta Branch, Rochester, NY) in addition to storage at Litron Laboratories.

9. References

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- Where applicable, GLP regulations for non-clinical laboratory studies as developed by the FDA (21 CFR 58). Please note that the computerized systems utilized for data acquisition, data analysis and report generation have undergone an internal validation guided by FDA GLP regulations. Litron is working towards 21 CFR part 11 compliance.
- Where applicable, Principles of GLP by OECD [C(97)186/FINAL].
- Where applicable, ISO 10993-3: Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicology (2003-10-15).
- Where applicable, ICH Harmonised Tripartite Guideline: Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, S2(R1), current *Step 4* version dated 9 November 2011.

10. Effective Date: January 01, 2014

APPENDIX N
SPERM ANALYSIS REPORT

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Sponsor

USARDECOM
ATTN: AMSRD-FE
Environmental Acquisition and Logistics Sustainment Program
Aberdeen Proving Ground, MD 21010

Study Title

Toxicology Study No.
Protocol No. 30-14-07-01
Contributing Scientist Report
Sperm Analysis of Rats (*Rattus norvegicus*) Exposed to Methylnitroguanidine

Author

Valerie H. Adams Ph.D.

Study Completed

September 2014

Performing Laboratory

U.S. Army Public Health Command
Portfolio of Toxicology
Health Effects Research Program
MCHB-IP-THE
Aberdeen Proving Ground, MD 21010

Laboratory Project ID

Protocol No. 30-14-07-01

Table of Contents

1 Summary

1.1 Objective

The objectives of this study were to determine the effects of oral subacute methylnitroguanidine (MeNQ) exposure on sperm parameters in the male rat.

1.2 Purpose

The purpose of this study is to provide environmental and occupational health information for a constituent of a new explosives formulation. This information is critical to the research, development, testing, and evaluation (RDT&E) of alternatives under the Environmental Quality Technology (EQT) program and is necessary for work unit program evaluation. Reproductive toxicity via testicular effects has been observed with other nitrogen containing constituents of munitions. An early measure of male reproductive toxicity is reduced sperm numbers. This report summarizes the findings of sperm analysis performed on rats dosed for 14 consecutive days with MeNQ.

1.3 Conclusions

When compared to the untreated control group, no changes in sperm quality parameters (motility and number) were observed in rats exposed to MeNQ. Although the 14 day study design is too short to identify slow acting or weak testicular toxicants, it is a suitable screen for potent testicular toxicants and toxicants that target the epididymis where a rapid loss of sperm would be detectable. Under the conditions tested, MeNQ is not a testicular or epidydimal toxicant.

1.4 Recommendations

MeNQ is not overtly toxic to the male reproductive system as measured by sperm quality parameters after 14 days of oral exposure. If further subchronic toxicity testing of MeNQ (i.e. 90-day) is recommended then sperm analysis should be included in the study design to identify latent effects not identified in the 14-day study.

2 References

References are listed in Appendix A.

3 Authority

The project sponsor for this study is Ms. Kimberly Watts, RDECOM, AMSRD-FE, Aberdeen Proving Ground, MD.

4 Background

TNT and RDX are two explosive compounds currently fielded by the U.S. Army. Both of these compounds have contributed to environmental contamination at manufacturing and training sites, causing range operations to be cut-back or terminated at some sites. Both RDX and TNT have negative health effects from both acute and chronic exposure and RDX is considered a potential carcinogen. MeNQ is an explosive compound that is currently under investigation as a component of a new energetic formulation consisting of four compounds that are expected to be less hazardous to humans and the environment.

An early step in the MeNQ assessment process is a 14-day oral toxicity test using rats. The 14-day study provides information regarding subacute toxicity and is a range finding method for 90-day subchronic testing. Testicular toxicity has been observed with other high nitrogen compounds such as TNT and NTO (Dilley et al. 1982; Levine et al. 1984; Levine et al. 1990; Quinn et al. 2014). Preliminary screening for

sperm quality in male rats exposed for 14 days to MeNQ is useful for identifying adverse effects that require further characterization in subsequent studies. By identifying unacceptable ESOH effects early in the acquisition process, unacceptable replacements can be identified and unnecessary budget expenditures can be greatly reduced.

5 Methods

The subacute study had 5 dose groups plus a corn oil control group and consisted of 10 male and 10 female rats per group and is more fully described in approved animal use protocol (30-14-07-01). A 3-day stagger start design was used and necropsies on male rats were performed on 3 consecutive days approximately 24 hours after the last MeNQ dose (i.e. 14th dose). Rats were euthanized via CO_2 and immediately necropsied. The right epididymis was first dissected from the reproductive system and then the distal cauda epididymis was further dissected and trimmed for analysis. The segment that was used for analysis was weighed (target weight 20-30 mg), minced with micro-scissors (5-12 cuts) and then placed into a 1.5 mL microcentrifuge tube containing 0.5 mL of prewarmed RPMI-1640 (Lot # AYK172851- exp APR 2015; Hyclone, Logan UT). The minced tissue was gently tamped with micro-pestle to release the epidydimal contents into the medium and incubated for 4 minutes at 37^{o}C. After the incubation, the tube was inverted once to mix the solution and an aliquot was removed and diluted 1:5 with warm (37^{o}C) RPMI-1640. Cauda epididymal sperm counts were determined using a computer-assisted sperm analyzer (TOX IVOS-CASA, Hamilton-Thorne, Beverly MA). A chamber of a rat toxicology slide (Leja® SC-100-01-02-A 100 Micron Counting Chamber, Spectrum Technologies, Healdsburg CA) was loaded with approximately 40 µL of the diluted sperm suspension and the slide was inserted into the sperm analyzer. For each chamber, the image analysis was performed in duplicate. The IVOS-CASA software reported the number (million/mL and million/g tissue) and percent of motile sperm and progressive sperm. Samples with very low or high counts were suitably diluted to accommodate the analytical parameters of the software.

Statistical analyses of the Million sperm/g tissue, percent motile and percent progressive sperm were performed using SigmaPlot 12.0. ANOVA analysis and Tukey's test were used if the data met variance and normality criteria. A Kruskal-Wallis One Way Analysis of Variance on Ranks was used where the data failed normality.

6 Results

For each terminal animal, sperm from a section of epididymides dissected at necropsy was evaluated for total sperm and sperm motility. The individual and summary data tables are provided in Appendices C and D, respectively. The descriptive statistics and statistical analysis are provided in Appendix E. A total of 59 rats were used for sperm analysis: Dose/number- 0/9, 100/10, 210/10, 415/10, 830/10, 1250/10. The range of million sperm per gram epididymal tissue was large (0-1028 M/g) and no statistically significant differences between dose groups were identified. Rat # 14-0661 had a small epididymis and no sperm. Rat # 14-0666 had a low sperm count of 30 M/g. These findings were not considered dose related.
The data sets for percent motile sperm and percent progressive sperm failed normality and were assessed with an ANOVA on Ranks. There were no statistically significant differences between the dose groups for either the percent motile or progressive sperm.

7 Discussion

The subacute toxicity of MeNQ was tested in rats. As a component of the main study, at necropsy a segment of the caudal epididymis from the on study male rats was used to assess sperm numbers, motility and progressivity. The time span of a 14 day study is insufficient to identify weak testicular toxicants; however potent toxicants and direct epididymal toxicants may be detected after 14 days of oral

exposure (Linder et al. 1992). Oral exposure to MeNQ for 14 days did not adversely affect the measured sperm parameters. When combined with testes weight data (absolute and normalized to brain weight), MeNQ was not a testicular toxicant at doses as high as 1250 mg/k-d over a 14-day dosing period. These results do not exclude the possibility that MeNQ may be a testicular toxicant over a longer exposure period.

8 Point of Contact

The contributing scientist report for sperm analysis was developed by Dr. Valerie H Adams, Biologist HERP.

APPENDIX A

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APPENDIX B

Sperm Analysis Individual Data

Date	Animal #	MeNQ mg/kg-day	sample 1				sample 2				Average		
			Total %	Motile %	Pro-gressive %	Million/ gram	Total %	Motile %	Pro-gressive %	M/g	Motile %	Pro-gressive %	Million/ gram
AUG 26 2014	14-0625	415	100	9	5	427.9	100	24	7	352.6	16.5	6	390.25
	14-0626	415	100	54	10	1036.6	100	40	10	1019.3	47	10	1027.95
	14-0629	210	100	31	6	920.4	100	40	9	835.9	35.5	7.5	878.15
	14-0630	210	100	32	17	755.5	100	38	15	802.2	35	16	778.85
	14-0637	1250	100	7	4	589.4	100	11	2	689.9	9	3	639.65
	14-0638	1250	100	10	0	232.4	100	27	4	240.4	18.5	2	236.4
	14-0639	0	100	68	13	438.9	100	54	13	362.2	61	13	400.55
	14-0640	0	100	83	21	878.6	100	67	16	732.1	75	18.5	805.35
	14-0645	100	100	39	14	366.5	100	46	19	380.5	42.5	16.5	373.5
	14-0646	100	100	0	0	318.5	100	1	0	313.5	0.5	0	316
	14-0663	100	100	0	0	221.9	100	0	0	208.7	0	0	215.3
	14-0664	100	100	88	30	548.9	100	85	29	459.8	86.5	29.5	504.35
	14-0667	415	100	74	34	256.9	100	71	36	242.4	72.5	35	249.65
	14-0668	415	100	67	16	434.9	100	49	11	344.2	58	13.5	389.55
	14-0675	210	100	56	21	226	100	68	19	262.1	62	20	244.05
	14-0676	210	100	81	17	525.8	100	76	19	506.5	78.5	18	516.15
	14-0679	830	100	5	2	406.2	100	4	1	375.1	4.5	1.5	390.65
	14-0680	830	100	95	41	570.6	100	90	28	541.5	92.5	34.5	556.05
	14-0687	0	100	28	5	420.6	100	37	9	428.4	32.5	7	424.5
	14-0688	0	100	81	28	499.2	100	80	29	493.7	80.5	28.5	496.45
AUG 27 2014	14-0627	1250	100	93	35	466.5	100	91	35	495.4	92	35	480.95
	14-0628	1250	100	95	22	360.6	100	78	28	319.8	86.5	25	340.2
	14-0633	415	100	88	34	616	100	87	34	559.1	87.5	34	587.55
	14-0634	415	100	87	34	484.8	100	84	32	448.2	85.5	33	466.5
	14-0635	0	100	77	23	590.3	100	83	21	716.3	80	22	653.3
	14-0649	210	100	78	27	375	100	80	27	380.5	79	27	377.75
	14-0650	210	100	79	20	566.6	100	71	20	632	75	20	599.3
	14-0653	100	100	87	32	474.3	100	86	34	527	86.5	33	500.65
	14-0654	100	100	3	0	243.3	100	2	0	234.5	2.5	0	238.9
	14-0655	830	100	88	36	570.4	100	90	36	553.5	89	36	561.95
	14-0656	830	100	95	37	502.2	100	87	38	489	91	37.5	495.6
	14-0661	830	100	0	0	0	100	0	0	0	0	0	0
	14-0662	830	100	84	43	362	100	82	45	402.9	83	44	382.45
	14-0671	1250	100	7	2	290.4	100	6	1	270.8	6.5	1.5	280.6
	14-0672	1250	100	77	27	336.1	100	77	26	376.1	77	26.5	356.1
	14-0673	0	100	86	14	212.9	100	91	13	201.3	88.5	13.5	207.1
	14-0674	0	100	9	3	253.9	100	7	1	240	8	2	246.95
	14-0681	415	100	39	23	336.1	100	40	6	314.9	39.5	14.5	325.5
	14-0682	415	100	43	17	235.1	100	48	21	276	45.5	19	255.55
	14-0683	210	100	71	17	372.8	100	69	20	362.8	70	18.5	367.8
	14-0684	210	100	82	19	224.8	100	76	19	207.2	79	19	216
AUG 28 2014	14-0631	1250	100	81	35	547.6	100	77	34	518.3	79	34.5	532.95
	14-0632	1250	100	75	27	349.5	100	77	26	389.4	76	26.5	369.45
	14-0641	100	100	88	38	522.3	100	88	37	525.4	88	37.5	523.85
	14-0642	100	100	19	7	260.3	100	14	5	228.3	16.5	6	244.3
	14-0643	415	100	75	23	456	100	75	29	447.1	75	26	451.55
	14-0644	415	100	85	32	369.4	100	87	36	423.6	86	34	396.5
	14-0651	1250	100	85	25	544.7	100	85	27	510.3	85	26	527.5
	14-0652	1250	100	49	16	363.3	100	44	18	339.8	46.5	17	351.55
	14-0657	0	100	90	34	461.3	100	93	36	369.8	91.5	35	415.55
	14-0658	0	100	88	31	638.2	100	86	31	594.4	87	31	616.3
	14-0665	210	100	86	26	558.3	100	86	25	557.1	86	25.5	557.7
	14-0666	210	100	1	0	27	100	1	0	32.4	1	0	29.7
	14-0669	830	100	88	33	794.8	100	80	27	540.6	84	30	667.7
	14-0670	830	100	18	6	273.2	100	12	5	234.4	15	5.5	253.8
	14-0677	100	100	87	27	438	100	85	28	442.6	86	27.5	440.3
	14-0678	100	100	84	31	493.1	100	93	34	504.4	88.5	32.5	498.75
	14-0685	830	100	90	30	465.9	100	90	30	419.4	90	30	442.65
	14-0686	830	100	80	32	337.3	100	70	21	271.8	75	26.5	304.55

APPENDIX C

Summary Data for Sperm Analysis

MeNQ mg/kg-day	N	Average			Standard Deviation		
		Percent Motile	Percent Progressive	Million/ gram	Percent Motile	Percent Progressive	Million/ gram
0	9	67.1	18.9	474.0	28.7	11.2	192.6
100	10	49.8	18.3	385.6	41.2	15.5	123.7
210	10	60.1	17.2	456.5	27.4	8.0	261.4
415	10	61.3	22.5	454.1	23.9	11.2	225.2
830	10	62.4	24.6	405.5	39.1	16.1	189.7
1250	10	57.6	19.7	411.5	34.3	13.1	127.5

APPENDIX D

Statistical Analysis of Sperm Parameters

Million sperm/gram epididymal tissue

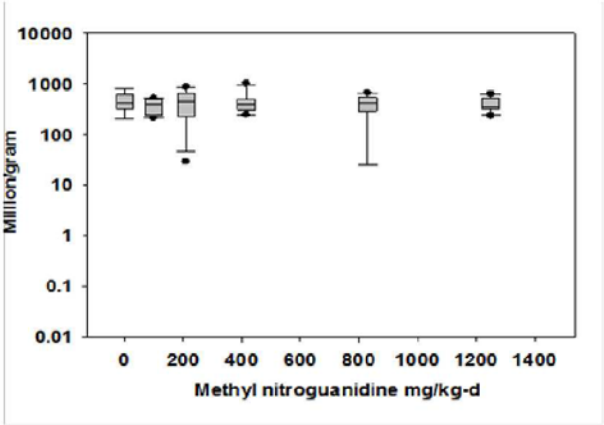
Normality Test (Shapiro-Wilk) Passed (P = 0.287); **Equal Variance Test:** Passed (P = 0.425)

Group Name	N	Missing	Mean	Std Dev	SEM
0-M/g	10	1	474.006	192.595	64.198
100 M/g	10	0	385.590	123.705	39.119
210-M/g	10	0	456.545	261.426	82.670
415-M/g	10	0	454.055	225.239	71.227
830-M/g	10	0	405.540	189.671	59.979
1250 M/g	10	0	411.535	127.537	40.331

Source of Variation	DF	SS	MS	F	P
Between Groups	5	59362.199	11872.440	0.318	0.900
Residual	53	1976321.760	37289.090		
Total	58	2035683.959			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.900).

Power of performed test with alpha = 0.050: 0.050. The power of the performed test (0.050) is below the desired power of 0.800. Negative results should be interpreted cautiously.



APPENDIX E

Sperm Percent Motility

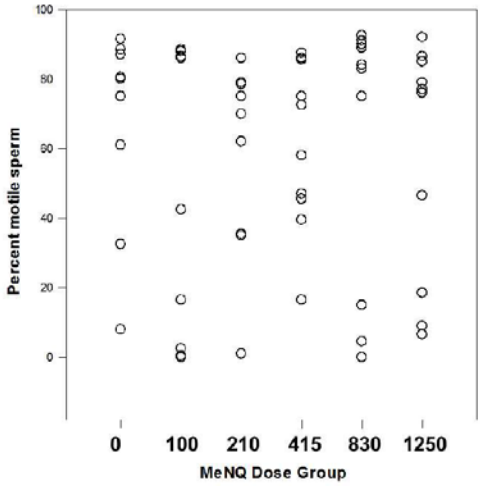
Normality Test (Shapiro-Wilk) Failed (P < 0.050)
Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
0-motile	10	1	80.000	46.750	87.750
100-motile	10	0	64.250	2.000	86.875
210-motile	10	0	72.500	35.375	79.000
415-motile	10	0	65.250	44.000	85.625
830-motile	10	0	83.500	12.375	90.250
1250-motile	10	0	76.500	16.125	85.375

H = 2.155 with 5 degrees of freedom. (P = 0.827)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.827)



APPENDIX E

Sperm Percent Progressive

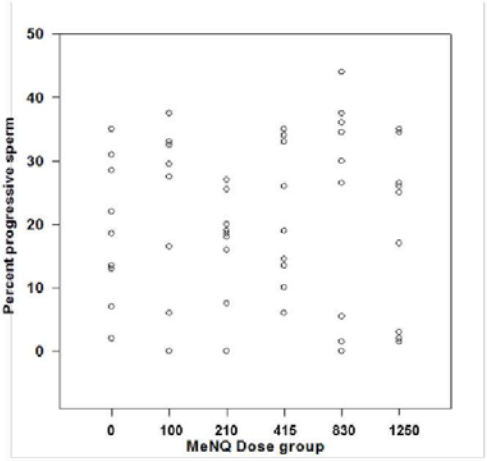
Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Kruskal-Wallis One Way Analysis of Variance on Ranks

Group	N	Missing	Median	25%	75%
0-progressive	10	1	18.500	10.000	29.750
100-progressive	10	0	22.000	0.000	32.625
210-progressive	10	0	18.750	13.875	21.375
415-progressive	10	0	22.500	12.625	34.000
830-progressive	10	0	30.000	4.500	36.375
1250-progressive	10	0	25.500	2.750	28.500

H = 3.131 with 5 degrees of freedom. (P = 0.680)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.680)



APPENDIX O
STATISTICAL EVALUATION OF 14-DAY RESULTS

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

Statistical analysis for organ weights, clinical chemistry, hematology, prothrombin times and the micronucleus assay are included in the raw data for this report. See below for the contributing scientist report for bodyweight analysis.

Fourteen Day Study on Effects of Acute and Subacute Oral Methylnitroguanidine Exposure to Rats
- Bodyweight Analysis -

I. Analysis Methods

Marginal Models were used to analyze the longitudinal bodyweight data. Marginal models were needed because multiple bodyweight measurements were taken from the same rat (day 0, 1, 3, etc.), i.e., a repeated measures design. Marginal models were used to properly measure the covariance structure (correlation) of bodyweights measured through time, giving the ability to account for within-rat variability. Multiple covariance structures were tested, but the one with the most accurate representation (lowest BIC) of the correlation of within-rat measurements through time was selected for the final model. SPSS V21 was used for data analysis.

Data was tested for the main effect of dose, time (measured in days), and a dose×day interaction. Statistical significance ($p<0.05$) was not found in testing the dose×day interaction or the dose main effect. Therefore, follow-up post-hoc testing was not necessary. This was true for both genders.

II. Summary

Dosing with Methylnitroguanidine had little to no effect on the bodyweights (and bodyweight gain) of male and female rats. All dose groups had linearly increasing bodyweight averages from day 0 (zero) to day 13, although male bodyweights increased with a higher trajectory.

III. Results

A. Males

The marginal model test revealed that dosage was not a significant factor affecting bodyweights in the day 0 through day 13 collection period for male rats ($p = .085$). There was also no presence of a significant dose×day interaction (difference in bodyweight trends) through the collection period ($p = .081$).

Day was found to be statistically significant ($p=.000$). This is not surprising considering the expected growth of young rats. The increasing bodyweight of all dose groups appears to be following a linear trend from day 0 to day 13.

The figure below shows that although variation among dose group bodyweight averages increased as the study progressed, the differences were still minimal and were not indicative of a true dose response pattern, i.e., the dosage amount was not a factor. It is evident that the 830 mg/kg-d dose group had a slightly higher starting bodyweight when compared to the other dose groups and steadily continued this pattern through the other measurement periods; however this difference was not enough to be considered significantly higher.

28 October 2014

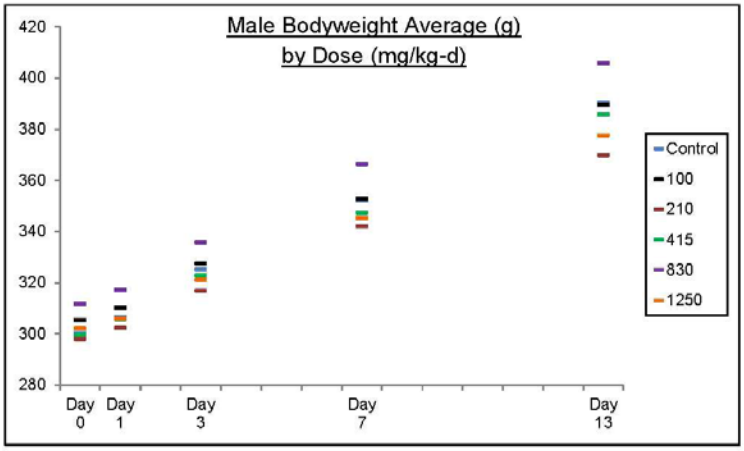


Table 1. Male Bodyweight Descriptive Statistics

Day	Dose (mg/kg-d)	Mean (g)	Std. Deviation	N
0	0	300.2	11.0	10
	100	305.3	17.4	10
	210	297.9	13.7	10
	415	299.6	13.6	10
	830	311.6	11.9	10
	1250	302.0	11.9	10
1	0	306.4	13.4	10
	100	310.2	17.3	10
	210	302.3	15.8	10
	415	305.7	15.9	10
	830	317.1	13.2	10
	1250	305.7	16.7	10
3	0	325.0	14.6	10
	100	327.4	20.2	10
	210	316.9	16.7	10
	415	322.7	17.8	10
	830	335.7	15.8	10
	1250	321.0	18.9	10
7	0	352.1	19.7	9
	100	352.6	23.0	10
	210	342.0	15.8	10
	415	347.3	20.4	10
	830	366.3	21.5	10
	1250	345.2	20.9	10
13	0	390.3	31.4	9

	100	389.4	32.6	10
	210	369.7	29.9	10
	415	385.7	25.9	10
	830	405.8	31.0	10
	1250	377.4	26.1	10

B. Females

Similar to males, the marginal model test revealed that dosage was not a significant factor affecting bodyweights in the day 0 (zero) through day 13 collection period for male rats (p = .873). There was also no presence of a significant dose×day interaction (difference in bodyweight trends) through the collection period (p = .339).

Day was found to be statistically significant (p=.000). This is not surprising considering the expected growth of young rats. The increasing bodyweight of all dose groups appears to be following a linear trend from day 0 to day 13.

The figure below shows that although variation among dose group bodyweight averages increased as the study progressed, the differences were still minimal and were not indicative of a true dose response pattern, i.e., the dosage amount was not a factor. In fact, in females, no single dose group consistently had the highest (or lowest) bodyweight average through all measurement periods.

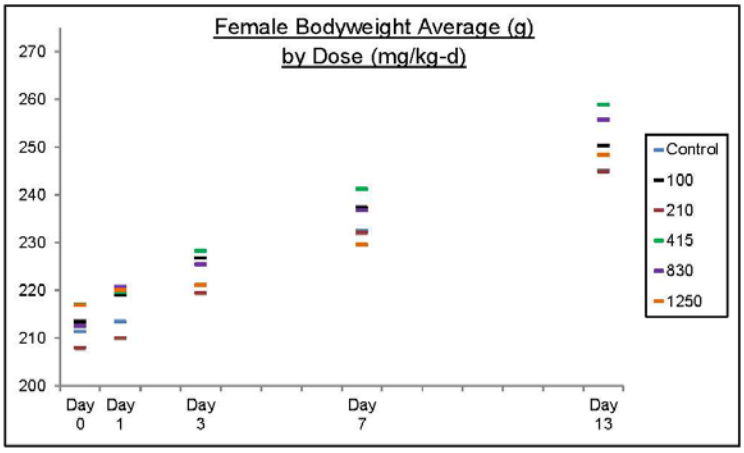


Table 2. Female Bodyweight Descriptive Statistics

Day	Dose (mg/kg-d)	Mean (g)	Std. Deviation	N
0	0	211.4	11.7	10

	100	213.4	7.0	10
	210	208.0	8.3	10
	415	217.0	15.2	10
	830	212.5	11.8	10
	1250	216.9	12.9	10
1	0	213.4	13.1	10
	100	219.1	10.2	10
	210	210.0	10.7	10
	415	219.6	15.1	10
	830	220.8	11.1	10
	1250	220.1	12.3	10
3	0	221.1	13.2	10
	100	226.7	12.1	10
	210	219.5	13.1	10
	415	228.2	14.1	10
	830	225.4	11.4	10
	1250	221.1	15.1	10
7	0	232.4	17.7	10
	100	237.3	14.2	10
	210	232.1	18.6	10
	415	241.3	19.1	10
	830	236.8	14.6	10
	1250	229.6	18.1	10
13	0	244.9	19.5	10
	100	250.3	18.6	10
	210	244.9	25.3	10
	415	258.9	21.4	10
	830	255.7	15.6	10
	1250	248.3	18.0	10

APPENDIX P
STUDY PROTOCOL AND MODIFICATIONS/DEVIATIONS

Protocol No: 30-14-07-01

Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*)

ANIMAL USE PROTOCOL
U.S. ARMY PUBLIC HEALTH COMMAND
ABERDEEN PROVING GROUND. MD 21010-5403

PROTOCOL TITLE: Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*).

PROTOCOL NUMBER: 30-14-07-01

DATE OF APPROVAL: 02 July 2014

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ACRONYMS:

AAALAC: Association for Assessment and Accreditation of Laboratory Animal Care
AIPH: Army Institute of Public Health
ALB: Albumin
ALK-P: Alkaline phosphatase
ALT: Alanine transaminase
ANCOVA: Analysis of Covariance
ANOVA: Analysis of Variance
AST: Aspartate transaminase
ASTM: American Society for Testing and Materials
AV: Attending Veterinarian
BRD: Biomedical Research Database
BUN: Blood urea nitrogen
CA: Calcium
CAS: Chemical Abstracts Service
CFR: Code of Federal Regulations
CHOL: Cholesterol
CHPPM: Center for Health Promotion and Preventive Medicine
CI: Confidence interval
CO_2: Carbon dioxide
CREA: Creatinine
CSD: Client Services Division
DI: Deionized
DNA: Deoxyribonucleic acid
DOD: Department of Defense
DTIC: Defense Technical Information Center
EDTA: Ethylenediaminetetraacetic acid
EMS: Ethyl methanesulfonate
EQT: Environmental Quality Technology
ESOH: Environmental Safety and Occupational Health
FEDRIP: Federal Research in Progress
FITC: Fluorescein isothiocyanate
GLP: Good Laboratory Practice
GLU: Glucose
IACUC: Institutional Animal Care and Use Committee
IAW: In accordance with
LD_{50}: Lethal dose 50%
LOAEL: Lowest Observed Adverse Effect Level

LOEL: Lowest Observed Effect Level
LS: Laboratory Sciences Portfolio
LTSS: Long Term Storage Solution
MeNQ: Methylnitroguanidine
MNA: Micronucleus Assay
NOAEL: No Observed Adverse Effect Level
NOEL: No Observed Effect Level
NRC: National Research Council
NSAID: Non-steroidal anti-inflammatory drug
NTIS: National Technical Information Service
OECD: Organisation for Economic Co-operation and Development
OEP: Ordnance Environmental Program
PE: Phycorythrin
PI/SD: Principal Investigator/Study Director
PHOS: Inorganic phosphate
RDX: hexahydro-1,3,5-trinitro-1,3,5-triazine
RPMI: Roswell Park Memorial Institute
SOP: Standing Operating Procedure
SSWP: Sequential Stage-Wise Probit
TNT: 2,4,6-trinitrotoluene
TOX: Portfolio of Toxicology
TP: Total protein
TRIG: Triglycerides
USAPHC: United States Army Public Health Command
USDA: United States Department of Agriculture
USEPA: United States Environmental Protection Agency
VMD: Veterinary Medicine Department

I. NON-TECHNICAL SYNOPSIS:

The purpose of this study is to assess the acute (one-time) and repeated-dose oral toxicity of methylnitroguanidine (MeNQ), a component of a new mixture formulated to replace 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) based fills in explosive formulations, in an effort to reduce environmental and/or occupational health hazards. This study will be comprised of two different experiments. In the first study, male and female rats will receive a single oral dose of MeNQ and be observed for 14-days for signs of toxicity to determine the oral LD_{50}. Data gathered from this experiment will guide the determination of dosing levels for the second experiment in which both female and male rats will be orally dosed with MeNQ daily for 14 consecutive days to determine the toxic effects of repeated exposures to the test material. Following the completion of each experimental endpoint, all surviving animals will be euthanized and selected tissues examined for any MeNQ related effects. The data gathered in this study will be used to compare toxicity of MeNQ to other compounds currently under consideration for use in explosive formulations.

II. BACKGROUND

II.1. Background:

TNT and RDX are two explosive compounds currently fielded by the U.S. Army. Both of these compounds have contributed to environmental contamination at manufacturing and training sites, causing range operations to be cut-back or terminated at some sites. Both RDX and TNT have negative health effects with both acute and chronic exposure, with RDX also being a potential carcinogen. MeNQ is an explosive compound that is currently under investigation as a component of a new energetic formulation consisting of four compounds, which are expected to be less hazardous to humans and the environment.

The Army EQT OEP is dedicated to finding replacements for TNT and RDX that will reduce or eliminate the health risks from environmental exposure and the adverse ESOH effects[1]. By identifying unacceptable ESOH effects early in the acquisition process, unacceptable replacements can be identified and unnecessary budget expenditures can be greatly reduced.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched: BRD, NTIS, PubMed, Web of Science, DTIC, FEDRIP

II.2.2. Date of Search: April 25, 2014

II.2.3. Period of Search: 1900-2014

II.2.4. Key Words of Search:
(methylnitroguanidine or N-methyl-N'-nitroguanidine or 4245-76-5 or nitroguanidine*) and (toxic or toxicity or no-observed-adverse-effect-level or no observed adverse effect level or loael or loel or noael or noel) and (oral or mouth) and (lethal dose 50 or ld50 or ld50 or ld-50 or median lethal dose or mld) and (rat or rats)

II.2.5. Results of Search:

IAW IACUC SOP 1.2 every effort to uncover literature relating to MeNQ toxicity was made by searching the above databases[2]. In the literature search, a total of 158 citations were found utilizing the above listed key words in the listed databases. One previously known study was found, which conducted a single dose limit test of 1000 mg/kg in 5 male and 5 female rats[3], as well as a technical report produced for the Air Force by contractors, written for the same study results[4]. As this was only a single dose test, it was not sufficient to calculate the LD_{50} value, slope or confidence intervals, nor did it conduct doses to the minimal limit of 2000 mg/kg. Of the other 156 citations, 15 were directly related to nitroguanidine or nitrosoguanidine, 33 to ethyleneglycol nitrates or methyl-phthalate based propellents, 43 involved the use of N-methyl-N'-nitro-nitrosoguanidine or N-ethyl-N'-nitro-N-nitrosoguanidine in gastric cancer research, 3 involved research with N-methyl-N-nitrosourea. The remainder of the citations was

related to policy, risk assessment, environmental policies or handbooks for the use of explosives in general. Based on this literature search, the present study is not a duplication of effort.

III. OBJECTIVE/HYPOTHESIS:

The objectives of this study are to 1) determine the oral LD_{50} of MeNQ, along with the 95% confidence interval and slope constant; and 2) to determine the health effects, including analysis of sperm and assessment of compound genotoxicity using the MNA (males only), of repetitive oral exposure to MeNQ in male and female rats.

IV. MILITARY RELEVANCE:

A functional, effective, quality engineered warhead formulation comprised of environmentally viable alternative substances can make a positive contribution to current and future Army readiness by being less toxic to the environment and human health. Through reduced environmental compliance constraints, a safer, more environmentally benign formulation can also increase life-cycle cost effectiveness. Current formulations that use TNT and RDX have contributed to environmental contamination at manufacturing and training sites, and have caused range operations to be curtailed or terminated at some locations. Costs for remediation of TNT- and RDX-contaminated sites are also significant, and consume resources that could be applied to other needs. It is imperative that the Department of the Army train its Soldiers in the same manner in which they fight, which requires the use of the same weapons. The development of weapons containing alternative/replacement energetics is a necessity. The acute and subacute toxicity tests proposed in this protocol can be used as a useful screening tool to provide support in developing less toxic munition alternatives to TNT and RDX[1].

V. MATERIALS AND METHODS

Test Article: This study will be conducted with MeNQ (see Table 1). The test material will be analyzed for purity prior to study initiation, if no certificate of purity is provided by the supplier. All concentration verification analysis of the dosing solutions (in DI water, drinking quality water, corn oil or methylcellulose), homogeneity of solution (if necessary) and stability analyses will be performed by AIPH Laboratory Sciences, Client Services Division, Method Development Section (SOP 801).

Table 1. Test Substance Chemical/Physical Properties

Name	Methylnitroguanidine
Synonym	MeNQ or 1-Methyl-3-nitroguanidine
CAS#	4245-76-5
Physical State	White powder
Molecular Formula	$C_2H_6N_4O_2$
Molecular Weight	118.1
Solubility	1.0E6 mg/L

V.1. Experimental Design and General Procedures:

In experiment 1, MeNQ will be administered by oral gavage in a single dose to determine the LD_{50}. In experiment 2, male and female rats will be dosed daily for 14 days by oral gavage at five different doses (derived by the SSWP method of the acute study). In the second experiment, a vehicle-only group will be included as a control. The study endpoint for both experiments will be mortality or euthanasia, either when the rats become moribund due to compound toxicity or 14-days after the onset of treatment, which is the end of the study. Blood and tissue samples will be collected at the end of the study for analysis of chemistry values and for histopathology, including analysis of sperm. In order to detect the potential genotoxicity of MeNQ in male rats, the potential for DNA damage will be measured using the erythrocyte MNA concurrent to the 14-day subacute study.

Table 2: Summary of Animal Use and Pain Category

Group	No. of Male Rats	No. of Female Rats	Pain Category
SSWP			
MeNQ	30	30	30C/30E
	TOTAL = 30	TOTAL =30	TOTAL = 30C/30E
Subacute Study			
Vehicle Control	10	10	20D
MeNQ Dose 1	10	10	20D
MeNQ Dose 2	10	10	20D
MeNQ Dose 3	10	10	20E*
MeNQ Dose 4	10	10	20E*
MeNQ Dose 5	10	10	20E*
MNA Control	5	0	5E*
	TOTAL = 65	TOTAL = 60	TOTAL = 60D/65E*
	TOTAL = 95	TOTAL = 90	GRAND TOTAL = 30C/60D/95E*

*If animals do not show any signs of distress, the pain category will be downgraded.
Pain categories were determined as follows:
B: Held, but not used on study
C: No pain or distress- It is expected that half of the acute study rats will not experience pain or distress.
D: Alleviated pain/distress with immediate moribund euthanasia or drug treatment (anesthesia/analgesics). Rats placed in this category were done so due to method of blood collection (cardiac puncture).
E: More than momentary pain/distress that cannot be alleviated. Rats placed in this category have the potential to experience toxic effects of compound treatment.

V.1.1. Experiment 1: LD_{50} Determination (Acute Study)

The primary objectives for Experiment 1 are to determine the oral LD_{50} and slope constant for MeNQ in the Sprague-Dawley rat, and to set dosage levels for the subacute (14-day) study. The methodology for this study will follow the ASTM stage-wise adaptive-dose approach, SSWP[5]. Oral dosing is described in section V.4.4.8.1.

This method proceeds in stages, where animals are fasted overnight, dosed via oral gavage and their responses observed in order to determine subsequent doses and animal numbers in subsequent dosing stages. Animals are observed post-dosing for a period up to 14-days, in which they are monitored for signs of toxicity, moribundity, and mortality. This period may be reduced to 24-48 hours for determination of dosages in subsequent stages if the PI/SD is confident in the survival of the animals beyond 48-hours. In the first stage, approximately five different doses of MeNQ will be selected, with doses spanning the entire expected dose response curve. In the absence of previously established values, 175 mg/kg may be used as a default starting value, with a progression of half-log dose intervals (3.2 dose progression factor), up to 7 animals may be used[6]. If there is historical toxicity data available, the numbers of animals used in the first stage may be reduced to 4 animals. The only published in vivo toxicity value for MeNQ is a single dose of 1000 mg/kg in Fischer 344 rats, in which no signs of toxicity were observed following this single dosage[3]. The limit dose of 2000 mg/kg was not tested. Based on these data, four rats will be used in the first stage, with each animal receiving a different oral dose, with the maximum dose at 2000 mg/kg. The dose intervals are typically spaced at 1/3 log (e.g., 61, 195, 625 and 2000 mg/kg). This utilizes fewer animals while still attempting to narrow the range of MeNQ toxicity.

Doses for the second stage of dosing will be based on the doses from the first stage where lethality is observed. In the SSWP methodology, 3-5 doses which bracket the lethal dose observed in the first stage will be used, with 2-3 animals utilized for each dose. A probit analysis of results from the previous stage(s) will be used to determine doses for each subsequent stage of dosing. This analysis uses the results from each stage to calculate the LD_{50}, 95% confidence interval, and slope of the dose response curve. Dosing will proceed until either the ratio of the 95% confidence interval divided by 2 times the estimated LD_{50} is less than 0.40 or 30 animals have been used, whichever occurs first. If no deaths are observed at the highest dose level (2000 mg/kg) in the first stage of dosing, a limit test will be conducted. In the limit test, five additional animals are dosed, of which a minimum of three must survive in order to determine that the LD_{50} is greater than the limit dose[7].

Bodyweights will be obtained prior to MeNQ administration, at least weekly during the observation period, and at termination. Note, animals may be weighed more frequently if clinical signs of toxicity are observed. Animals which have been dosed will undergo a thorough physical examination by study personnel at a similar time each day during the 14-day observation period. This will consist of each rat being removed from its home cage, handled and observed. Observations will include, but are not limited to, evaluation of skin, fur, eyes and mucus membranes, respiratory and circulatory effects, autonomic effects, including salivation, central nervous system effects, including tremors and convulsions, changes in activity levels, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or abnormal behavior. All data related to the observation of the rats will be detailed and documented in the study records by study personnel.

The LD_{50} determination will use up to 30 female and 30 male Sprague-Dawley rats (**n=60,** see Table 2). If fewer than 30 rats of each gender provide sufficient results, the remaining animals will be humanely euthanized per VMD SOP 002[8], transferred to a training protocol if requested by the Attending Veterinarian, or transferred to another study protocol. All surviving dosed animals will be euthanized and undergo gross necropsy at the end of the 14-day observation period. Select tissues (to include at minimum brain, heart, lungs, liver, spleen, and thymus) may be removed, weighed and preserved in 10% formalin. Dosed animals that are found moribund or are determined to be too sick by the vet or study staff will be humanely euthanized and undergo gross necropsy at the time of euthanasia.

V.1.2. Experiment 2: 14-day Repeated Dose Test (Subacute Study)

The primary purpose of the 14-day study is to determine any adverse effects from short-term repetitive oral exposures to MeNQ. If the results from this study suggest that significant effects do occur, a subchronic study may be recommended. The study will consist of 5 dosing groups and a vehicle control, consisting of 10 males and 10 females for each dose group, as well as a MNA negative/positive control group consisting of 5 males (**n= ((10+10)x6)+5=125,** see Table 2). Animals will be assigned to dose groups, with animals stratified according to their weight following a bodyweight determination one day prior to dosing initiation. Test compound animals will be dosed daily via oral gavage with MeNQ in the appropriate vehicle (as determined by the acute study; e.g., distilled water, drinking quality water, corn oil or methylcellulose) for 14-days (without fasting). Dose selection will be based on the results of the LD_{50} study (e.g. 0.75x, 0.5x, 0.25x, 0.125x, 0.0625x the LD_{50}). The appropriate amount of MeNQ to be delivered to each animal will be calculated based on the most recently collected weight for each animal. The vehicle control group will receive a volume equivalent to the highest exposure group. For oral dosing, the maximum volume will not exceed 10 mL/kg[9, 10]. The MNA negative/positive control group is dosed as described in section V.4.4.8.1 and V.4.4.8.2.

All rats will be monitored for body weight changes and clinical signs of toxicity throughout the study. Weights will be taken on day -3 (for general health check), -1 (randomization), 0 (starting weight and first dosing day), plus +3, +7, +13 (final weight before fasting; last dosing day) and +14 (terminal weight). Clinical observations for signs of toxicity will be made on a daily basis. Records will be kept in standard USAPHC laboratory notebooks and/or three ring binders. Daily records will be kept on survival and clinical signs collected on animals during the study. Weights will be recorded on scheduled days concurrently with clinical signs. Individual animal weights may be taken more frequently if signs of toxicity are observed. The 14-day study is a stagger start over a three day period, per sex. The 4 MNA negative/positive control rats are subdivided prior to dosing into 2 groups of 2 and dosing is scheduled so that their necropsy dates coincide with the subacute study necropsy dates (2 animals/necropsy day)[11]. These animals serve as internal controls for the MNA assay.

On the Thursday prior to the initiation of necropsy (5-6 days prior to necropsy start), the male negative/positive MNA controls (**n=5**, see Table 2) will be biosampled from the saphenous or lateral tail vein [12, 13], which will serve as the negative control for the MNA study. On study day 14, 5 male rats from the three highest dose groups with no mortalities will be biosampled from the saphenous or lateral tail vein for the MNA.

All animals will be fasted overnight (< 24 hours) prior to necropsy, anesthetized with CO_2 gas, bled, euthanized with CO_2 and necropsied. Fasting is necessary in order to reduce variability in clinical chemistries due to food intake. At minimum, the following tissues will be harvested, weighed and preserved in 10% formalin: brain, heart, kidneys, lungs, liver, spleen, and thymus. The following organs will be preserved in Davidson's for 24-hours, and then transferred to 70% ethanol: ovaries, uterus, testes, and left epididymis. The right epididymis will be dissected and a portion of the caudal epididymis will be used for sperm analysis. Additional organs and tissues may be collected, if determined to be necessary based on necropsy observations. All gross pathology changes will be recorded on CHPPM Form 333 or equivalent GLP compliant documentation system. Training records of necropsy personnel will be verified and the names of personnel participating in necropsy will be documented. A table containing the training record information for personnel performing necropsy procedures will be part of the study records. Full histopathology of at least the previously listed organs will be performed for all surviving high-dose and control animals. Organs demonstrating treatment-related changes may also be examined in the lower dose groups. All gross lesions will be subjected to histopathological evaluation.

Clinical chemistry and hematology will be conducted on all viable samples at the end of the study. This is in accordance with TOX SOP 005 Clinical Chemistry Analysis of Blood Specimens[14] and TOP SOP 013 Cell-Dyn3700 Hematology Analyzer[15]. Cardiac blood will be collected as described in V.4.4.3, Biosamples. A portion of blood will be transferred to a 1.3 mL EDTA microtube and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and a five-part leukocyte differential. Remaining blood will be transferred to the appropriate number and type of microtubes (e.g. serum-gel and sodium citrate) and evaluated for the following chemistries: prothrombin, ALB, ALKP, ALT, AST, BUN, CA, CHOL, CREA, GLU, PHOS, TP, TRIG and electrolytes, and/or other chemical parameters that may be affected by MeNQ.

V.1.3 Study Conduct:

The study described will be conducted in a manner consistent with the principles of GLP regulations in the Toxic Substances Control Act: 40 CFR792, plus amendments[16]. The investigators and technicians will adhere to *The Guide for Care and Use of Laboratory Animals*[10].

V.1.4 Study Time Frame: Estimated initiation date is July 2014. Estimated experimental completion date is September 2014.

V.2. Sample Size Evaluation, Data Analysis Plan, and Archiving of Data:

With the exception of clinical chemistry, hematology and genotoxicity data, all data will be recorded in the laboratory notebook or on data sheets. The data from the SSWP will be analyzed according to the methods of Feder et al.[17, 18] in order to obtain an estimated LD_{50} value, 95% confidence interval, and slope. Note, an LD_{50} and slope are not calculated if the LD_{50} is above the limit dose of 2000 mg/kg. Sample sizes for the acute and subacute studies were selected in accordance with USEPA Health Effects Testing guidelines[7, 19]. These sample sizes have been widely used and have been demonstrated to provide adequate statistical power in these methods. MNA control sample size is in accordance with OECD Guideline 474.[11] Continuous data will be analyzed using a one-way ANOVA with dose group as the main effect. Organ weights will be analyzed using ANCOVA with terminal body weight as the covariate. When statistically significant main effects are observed ($p<0.05$), an appropriate post hoc test will be used to compare pairs of dose groups and dose groups to the control group. Variance equality will be determined by Levene's test. Data will be tested for normality and may be transformed appropriately prior to ANOVA/ANCOVA, or analyzed using a nonparametric Kruskal-Wallis test. Non-parametric analysis will be the method of last resort since it does not allow analysis of co-variation. The choice of statistical software will be at the discretion of the PI/SD and AIPH statistician. For all tests $\alpha = 0.05$ is the level of significance.

Records will be kept in standard USAPHC laboratory notebooks and/or three ring binders. Daily records will be kept on survival and clinical signs collected on the animals after dosing occurs. Procedures for preparation of any euthanasia solution, drug administration, animal bleeds, observation logs, morbidity/mortality logs, etc. will be stored with the study records. All post mortem procedures not listed in this protocol will be documented in the study records and kept with the study raw data. Records pertaining to this study will be made available to oversight organizations such as the USEPA and the IACUC[10, 16]. The protocol, protocol amendments, raw data, statistical analysis, tabular calculations, and graphic analysis of the data will be saved with the study records. Additionally memoranda to the study file, study logs, signature logs, final reports, final report amendments, and test and control articles will be archived at USAPHC.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered:

The objectives of this study are to determine the adverse health effects of oral exposures of MeNQ in the rat. There are no appropriate animal substitutes (e.g., computer models, tissue/cell cultures) that simulate the pharmacokinetics and pharmacodynamics of *in vivo* animal exposure. No non-animal alternative would provide the necessary toxicological information provided by this study. Therefore, it is necessary to perform these studies in an animal model.

V.3.2. Animal Model and Species Justification:

Sprague-Dawley is the strain of rat that has been historically used for oral toxicity studies by USAPHC TOX and is the recommended species due to an extensive historical database. Both male and female rats will be utilized during both the acute and subacute portions of the study, in order to determine if there are gender differences in sensitivity to MeNQ, in addition to testing for effects on the function of the epididymis in males during the subacute portion.

V.3.3. Laboratory Animals

V.3.3.1. Genus species: *Rattus norvegicus*

V.3.3.2. Strain / Stock / Breed: Sprague-Dawley

V.3.3.3. Source / Vendor: Charles River Laboratories (USDA 14-R-0144) or other USAPHC approved animal vendor.

V.3.3.4. Age: Upon receipt: Acute: 7-11 weeks
14-Day: 8-10 weeks

V.3.3.5. Weight: Appropriate for age

V.3.3.6. Sex: Male and female (nulliparous and non-pregnant)

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (by species): 185

V.3.5. Refinement, Reduction, Replacement (3 Rs):

V.3.5.1. Refinement:

"In accordance with VMD SOP 004[20], standard rat enrichment will be implemented in this study. Animals will be socially housed and provided a form of environmental enrichment (e.g., nylabones, rodent retreats) throughout the study." Animals on this study will be handled on a frequent basis, except where necessary for the study protocol (MNA negative/positive controls). Animals will be considered for early removal from this study as described in section V.4.5. Animals will be deeply anesthetized prior to collecting blood for cardiac puncture and then immediately euthanized. Animals will be considered for early removal from the study based on clinical signs of morbidity.

V.3.5.2. Reduction:

1) The SSWP method uses fewer animals than the up and down LD_{50} estimation[7] while providing quantitative estimates of median lethality, slope, and confidence intervals.
2) If a dose of 2,000 mg/kg body weight does not cause mortality or morbidity in the first round of acute dosing, then round two of acute dosing will revert to an USEPA limit test and thus use fewer animals to estimate an LD_{50}.
3) The MNA positive controls will serve as their own negative controls through early saphenous or lateral tail vein sampling prior to dosing. This reduces the number of animals necessary by half, and provides a better control for the test itself.
4) Incorporating the MNA genotoxicity test with the subacute experiment reduces the need for initiating a separate genotoxicity study.
5) Allowing tissue sharing may also reduce the need for other animal studies to collect additional tissues.

V.3.5.3. Replacement:

No non-animal alternatives are known to exist that will provide the required toxicological information for MeNQ. At this time, there are no non-animal alternatives that can fully replicate the complex processes that occur within an intact mammalian organism. The use of the rat is the most preferred animal model according to the USEPA Health Effects Test Guidelines.

V.4. Technical Methods:

V.4.1. Pain / Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column B: __0__ (animal #)

V.4.1.1.1.2. Column C: __30__ (animal #)

V.4.1.1.1.3. Column D: __60__ (animal #)

V.4.1.1.1.4. Column E: __95__ (animal #)

V.4.1.2. Pain Relief / Prevention

V.4.1.2.1. Anesthesia / Analgesia / Tranquilization:

Animals will be anesthetized with CO_2 prior to cardiac blood collection. Animals will be brought to the necropsy room in home cage or transport cage. The stainless steel lid will be placed on the cage. The CO_2 tank will be turned on, the regulator opened to approximately 1/4 to 1/2 turn, and the flow meter set to 5 L/min. Animals will remain in the cage until they are recumbent, with shallow breathing patterns. Once recumbent, a

toe or space between the toes will be pinched to assess appropriate depth of anesthesia. If there is no response to toe pinch, the animal will be removed and blood collected (as described in V.4.4.3.). Upon completion of blood collection the animal will be returned to the cage and euthanized IAW VMD SOP 002[8].

V.4.1.2.2. Pre- and Post-procedural Provisions:

In the acute portion of the study (Experiment 1), rats will be fasted overnight prior to dosing as per USEPA Acute Oral Guidelines[7]. Study personnel will be responsible for removing feed from the cage and documenting appropriate information. Fasting will not exceed 20 hours; for the acute study, feed bins will be returned approximately 3 hours after dosing by study personnel.
In the 14-day study (Experiment 2), rats will be fasted overnight prior to necropsy, with study personnel responsible for removing feed from the cage and documenting appropriate information. In both studies, a physical examination will be made at least once each day during all phases of each study. Observations will be detailed and carefully recorded in the study records, the details of which are noted in V.1.1. Animals will be euthanized when appropriate (as described in section V.4.5. "Study Endpoint"). Rats will be observed for the first 30 minutes after dosing for signs of respiratory distress or immediate toxicity; rodent retreats may be temporarily removed to allow for observation without disturbing the animals. If no clinical signs are observed after the first 30 minutes, then the rats will be observed again approximately 4 hours later following dosing with the possible exception of non-duty days (weekends/holidays) during the 14 day subacute study based on a weight-of-evidence approach (e.g. lack of general toxicity or clinical signs) and in consultation with the AV (see Section V.5.2.1).

V.4.1.2.3. Paralytics: N/A

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Source(s) Searched:

NTIS, PubMed, Web of Science

V.4.1.3.2. Date of Search: April 25, 2014

V.4.1.3.3. Period of Search: 1900-2014

V.4.1.3.4. Key Words of Search:

(methylnitroguanidine or n-methyl-n'-nitroguanidine or 1-methyl-3-nitroguanidine or 4245-76-5) and (oral or mouth) and (lethal dose 50 or LD 50 or LD50 or LD-50 or median lethal dose or mld) and (toxicity or no-observed-adverse-effect-level or no observed adverse effect level or LOAEL or LOEL or NOAEL or NOEL) and (pain or distress or * or refin* or reduc* or replac* or artificial or vitro or culture or tissue or cell or organ or insect or arachnid or invertebrate or fish or mollusc or cephalopod or simulat*

or digital or interactive or mannequin or manikin or model or cardiac or blood or cardiac puncture)

V.4.1.3.5. Results of Search:

A total of 13 citations were found using the above search terms in the listed databases. All but one referred to N-methyl-N'-nitro-N-nitrosoguanidine as a model for carcinogenic induction in rodents. The one article that did refer to MeNQ is the previously mentioned limit dose study [3], which did not address alternatives. No validated *in vitro* tests for acute and 14-day oral toxicity are currently available.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification:

The nature of the study precludes the use of totally painless procedures. The toxicity of the compound of interest is unknown and if toxic, the mechanism of toxicity is unknown. An attempt to alleviate pain or distress by the administration of anesthetics, analgesics, or other drugs may alter the manifestation of the toxic responses and/or interfere with the mechanism of toxicity. Typical pain relievers such as opiates and NSAIDs as well as anesthetics have the ability to mask certain toxic signs that may be observed due to the administration of the test compound, especially those signs resulting from pain or distress. Additionally, certain side effects such as alterations in blood chemistry and hematology may arise from the use of these drugs and could be misinterpreted by the investigator as clinical signs caused by the test material.

The observation of the onset, duration, and/or reversibility of toxic signs is critical to mechanistic interpretation. "Toxic signs" are defined in VMD SOP 016[21]. The minimal number of animals needed for statistical significance will be used. The final number of rats in each pain category will be reported to the IACUC annually and at the completion of the protocol. To minimize distress, any animal defined as moribund (see V.4.5) will be euthanized with CO_2 in accordance with VMD SOP 002[8].

V.4.2. Prolonged Restraint and Restraint Methods:

V.4.3. Surgery

V.4.3.1. Pre-surgical Provisions: N/A

V.4.3.2. Procedure: N/A

V.4.3.3. Post-surgical Provisions: N/A

V.4.3.4. Location: N/A

V.4.3.5. Surgeon: N/A

V.4.3.6. Multiple Survival Operative Procedures

V.4.3.6.1. Procedures: N/A

V.4.3.6.2 Scientific Justification: N/A

V.4.4. Animal Manipulations

V.4.4.1. Injections: N/A

V.4.4.2. Use of Non-pharmaceutical-grade chemicals:

MeNQ is not available in a pharmaceutical-grade composition. It is under investigation as described in the objectives section (III) of this protocol.

V.4.4.3. Biosamples:

Animals in the acute study will not be biosampled. In the 14-day study, blood will be collected by a terminal intra-cardiac blood draw. Animals will be deeply anesthetized with CO_2, blood drawn (0.5-7 mL) using an 18-21 gauge, 1 -1.5 inch needle, as outlined in VMD SOP 015[12]. Biosampling will be promptly followed by euthanasia via CO_2. For the MNA portion of the subacute experiment, two saphenous or lateral tail vein samples will be taken from the controls (4-8 days apart, 60-120 microliters each, section V.4.4.8.2)[12, 13]. The initial sample will collect 60-120 microliters of blood; additional blood flow will be stemmed with appropriate pressure at the sampling site. The second sample from controls, and the only sample from dosed animals will occur just prior to CO_2 anesthesia on the day of euthanasia and will be performed in accordance with VMD SOP 002[8] with the following exceptions: appropriately sized lancets or needles may be used to puncture the vein, aids to visualize the vein (shaving, alcohol and/or puralube) are optional, manual restraint will be used, and the collection tubes are described in V.4.4.8.2.

V.4.4.4. Adjuvants: N/A

V.4.4.5. Monoclonal Antibody (MAb) Production: N/A

V.4.4.6. Animal Identification: Individual animals will be identified by cage card and tail markings (indelible ink) according to VMD SOP 014[22].

V.4.4.7. Behavioral Studies: N/A

V.4.4.8. Other Procedures:

V.4.4.8.1 Oral Gavage:

All oral dosing will be administered using an oral gavage needle (e.g. 16 – 18 gauge x 2 inches) fitted to a 1 – 10 mL syringe. The maximum volume is not to exceed 10 mL/kg.

[10]. The materials planned for oral administration include MeNQ, EMS (for control rats in the MNA) and vehicle control. If a dose volume greater than 10 mL/kg is necessary, the total dose will be split into smaller equal doses (preferably two doses) to be administered at least 4-hours apart within a 24-hour period. In order to gavage the rat, the animal will be restrained by placing the index and middle fingers on either side of the rat's neck, with the remainder of the hand supporting the body. Alternatively, the rat may be "scruffed" by pinching the skin at the base of the neck between the thumb and forefingers, which will immobilize the head, neck and torso of the rat or through a modified version of either of these. The needle will be determined to be of appropriate size by visually assessing that the hub-to-bulb distance spans the length between the mouth and the last rib of the rat. Prior to needle insertion, the head will be tilted back with the index and middle fingers to allow for insertion of the gavage needle into either the side or top of the mouth. The needle is gently advanced down the esophagus until the hub of the gavage needle is at the opening of the animal's mouth. The material will be administered with a smooth, steady depression of the syringe plunger and the needle withdrawn. If any resistance is felt during the gavage procedure, the needle will be removed and the animal briefly released before attempting the procedure again. Once the material has been administered, the animal will be observed for acute signs of maladministration (e.g., gasping, respiratory distress, etc.) and euthanized when appropriate.

V.4.4.8.2 Micronucleus Assay:

Biosampling for the MNA will be from the three highest dose groups without mortality, the vehicle control and five MNA control animals (**n=25 animals**, see Table 2). The assay will be conducted using the MicroFlow Basic Kit® (Litron Laboratories, Rochester, NY) following manufacturer's instructions. In brief, 60-120 µL of blood will be used for analysis as described in V.4.4.3. "Biosamples." It is most important to consider that blood be free flowing and collected in syringes/tubes/pipette tips coated with the anticoagulant solution provided in the kit, and that no more than 60-120 µL of blood are collected per 350 µL of anti-coagulant. Following collection, tubes/syringes will be capped and inverted several times to mix the blood and anti-coagulant solution. This mixture can be stored at room temperature for up to 5 hours prior to fixation, or it may be refrigerated for up to 24 hours.

To fix the samples, methanol will be used. Methanol must be kept in an ultracold (-75 to -85 °C) freezer. In order to prevent methanol from warming, the fixation process will be performed quickly (less than one minute per sample) and within reach of an ultracold freezer. The following procedure will be followed for sample fixation. Immediately prior to fixation, the tube containing the blood sample will be briefly vortexed to ensure a homogeneous solution. With the use of a pipettor, 180 µL of the sample will be transferred to correspondingly labelled 15 mL tube (s) (two replicate samples per rat) containing 2 mL of ultracold methanol. The tube is recapped, briefly vortexed (3-5 seconds) and immediately transferred to the -75 to -85 °C freezer for storage until analysis by flow cytometry or transfer into LTSS. Samples moved into LTSS will be maintained at -75 to -85 °C until sent to Litron Laboratories for analysis.

The samples will be sent to Litron Laboratories (the kit manufacturer) for flow cytometric analysis. They describe their method of analysis as follows: aliquots of blood samples are washed to remove LTSS, resuspended and treated with RNase A, which is followed by treatment with specific surface marker antibodies conjugated with FITC and PE. Anti-CD71-FITC (detects immature red blood cells = reticulocytes), anti-CD61-PE (detects platelets) and propidium iodide (detects DNA) fluorescence signals will be detected in the FL1, FL2 and FL3 channels, respectively. The stop mode will be set so that 20,000 high CD71-expressing cells will be analyzed per sample. In the event of bone marrow toxicity, the number analyzed may be reduced according to Litron's SOPs. The proportion of immature erythrocytes among total erythrocytes will also be determined to provide an index of cytotoxicity. Micronucleated reticulocytes will be identified as those that show both CD71 and propidium iodide-associated fluorescence. The data collected from the micronucleus assay will be expressed as a percentage of reticulocytes with micronucleus (%MN-RET) and the percentage of total erythrocytes that were immature (%RET) in each sample.

During the subacute portion of the MeNQ study, 4 additional male rats will be included as concurrent joint negative and positive controls for the genotoxicity experiment. In brief, the positive control animals will serve as their own negative control, by obtaining a blood sample prior to treatment with a genotoxic agent (e.g. EMS @ 200 mg/kg; CAS 62-50-0). In order to get a negative control sample, the 4 male rats will be left untreated and will not undergo oral gavage, until saphenous sample collection (60-120 µL blood) on the Thursday prior to the start of necropsy as described in section V.4.4.3. The rats will then receive EMS treatment in an appropriate vehicle (e.g. 0.5% weight by volume sodium carboxymethylcellulose solution, polyethylene glycol 200, water, or corn oil; maximum volume 10 mL/kg) three times: approximately 48-hours, 24-hours and 4-hours prior to blood collection via saphenous venipuncture as described in section V.4.4.3, followed by CO_2 euthanasia (VMD SOP 002). The timing of the dosing for the EMS treated animals is staggered to coincide with the repeated dose subacute study such that euthanasia will be conducted on the same days as the subacute study.

V.4.4.8.3 Sperm Collection and Analysis

Caudal epididymal sperm counts will be determined using a computer assisted sperm analyzer (TOX IVOS-CASA). After removal, trimming, and weighing, one epididymis will be further trimmed to select the caudal portion and re-weighed. The trimmed caudal section will be placed in a suitable container containing a measured volume of RPMI-1640 medium warmed to 34-37 °C and minced using a scalpel or scissors to release sperm. The preparation will be incubated for 15 minutes at 34-37 °C, gently mixed to uniformly suspend the sperm, and an aliquot will be transferred to a new vessel and diluted in medium. A chamber of a rat toxicology slide (Leja® or Hamilton Thorne) will be loaded with approximately 20 µL of the diluted sperm suspension and the slide loaded into the sperm analyzer. For each chamber, the image analysis will be performed in duplicate. The IVOS-CASA software will count and analyze the sample and report the number and percent of sperm, motile sperm, and progressive sperm.

The data will be expressed as millions of sperm per ml of suspension and millions of sperm per gram caudal epididymis. Based on the IVOS-CASA results, a further assessment of morphology may be conducted. From the diluted sperm suspension, a small sample will be placed on a slide and can be viewed either as a wet preparation or the slide can be air-dried. Samples may be stained with Eosin Y, but a variety of stains are acceptable as long as they allow appropriate viewing of the sperm. The samples will be viewed with a light microscope at a magnification of 400X and at least 200 spermatozoa per sample classified as either normal (both head and midpiece/tail appear normal) or abnormal (e.g., fusion, isolated heads, and misshapen heads and/or tails)[23].

V.4.4.9. Tissue Sharing: Tissues from these experiments will be made available to collaborators and other USAPHC or DoD personnel with PI/SD and/or AV coordination, as long as no deviation from the protocol is required for collection.

V.4.5. Study Endpoint:

The study endpoint for the acute study is intervention euthanasia of moribund animals, study-related mortality or euthanasia following an observation period which is not to exceed 14-days. The study endpoint for the subacute study is intervention euthanasia of moribund animals or euthanasia on day 14 of the study (the day following the final administration of MeNQ).

The following clinical signs will be considered in deciding to remove an animal from study and administering euthanasia: prolonged impaired ambulation which prevents animals from reaching food or water; weight loss ($\geq$ 20% body weight as compared to controls) plus one other clinical sign or excessive weight loss ($\geq$ 25% body weight as compared to controls); prolonged labored breathing; unabated seizure activity lasting longer than 1 hour; inability to urinate or defecate for greater than 24 hours; or a prolonged (greater than 1 hour) inability to remain upright. The AV will be consulted to evaluate animals presenting clinical signs that the PI/SD has not euthanized immediately upon reaching the above early end point criteria. The AV and PI/SD will determine if euthanasia is indicated for these animals[8].

The time at which toxicity signs appear, their duration, and the time to death are important, especially if there is a tendency for deaths or morbidity to be delayed. At the end of the observation or dosing period, all surviving animals will be euthanized by CO_2 (surviving animals from the 14-day subacute study will first be deeply anesthetized for cardiac blood sampling) and necropsied.

Any animals not used in the acute study will either be transferred to another approved protocol or euthanized as described in section V.4.6.

V.4.6. Euthanasia:

Rats on study will be euthanized in the necropsy suite by CO_2 asphyxiation followed by thoracotomy or immediate necropsy with perforation of the diaphragm to ensure death in accordance with VMD SOP 002[8]. Moribund animals will be euthanized with CO_2 in accordance with VMD SOP 002[8]. The AV will be consulted to evaluate moribund animals unless the PI/SD plans to immediately euthanize the animal. The PI/SD or study personnel will perform the euthanasia.

V.5. Husbandry & Veterinary Care:

V.5.1. Husbandry Considerations:

Rats utilized for the acute study will be fasted overnight prior to dosing and up to 4 hours after dosing (no more than 20 hours) as per USEPA Acute Oral Guidelines[24]. All 14-day study animals will be fasted overnight prior to necropsy. Otherwise, all animals on study will be provided certified rodent chow *ad libitum*. Rat chow will have been certified that it is free of contaminants by manufacturer. Animal rooms will be maintained at a constant temperature range of 64-79 °F and a humidity range of 30-70% with a 12-hour light/dark cycle. Cage sanitation is checked at least once daily by animal care staff. Animals will be housed in plastic, solid-bottom shoebox cages (size appropriate for the body weight of the rat). Detailed husbandry practices and animal room sanitation schedules are contained in VMD SOP 008.001[25]. Rats will be same-sex pair-housed within treatment group for the 14-day study. Animals may be separated at the discretion of the PI/SD/study personnel/AV if the animals display signs of extreme aggression with evidence of fighting (e.g., bite marks, hair pulling, etc.). Animals utilized for the acute study will be singly housed following dosing as a precaution for the unknown toxicity of the compound (See V.5.1.3.). Animals which show no signs of toxicity during the first 7 ± 2 days of the observation period may be pair-housed again at the discretion of the PI/SD and the AV. Pair-housed animals that show signs of delayed toxicity will be returned to single housing. Animals will be acclimated upon arrival at the animal facility for no less than 5 days. Body weight and observation data may also be collected for rats by study personnel during the acclimatization period in an attempt to more accurately monitor the health status of the rats in preparation for their use on study. They will not be handled or weighed by study personnel within the first 24-hours of arrival into the animal facility. The animal facilities are fully AAALAC accredited. Detailed husbandry practices and animal room sanitation procedures are contained in VMDSOP 008[25].

V.5.1.1. Study Room: Studies will be conducted at the USAPHC animal facilities, Bldg E-2100 or Bldg E-2101, study room as assigned.

V.5.1.2. Special Husbandry Provisions:

Animals will be pair-housed (same sex) during the acclimatization period for all tests. Animals will be singly housed for the acute portion of the study (See V.5.1.3.) Animals will be pair housed (same sex) during the 14-day study for all tests unless behavioral changes (e.g. aggression or hyperactivity) warrant single housing. When animals are

being fasted, PI/SD or study personnel (or Vet Med staff when directed to do so) will remove the food hopper no later than 1800 and no earlier than 1400 the day prior to dosing or necropsy. Animals on the acute study will be fasted for no more than 12 to 18 hours with the feeder returned 3-4 hours following dosing. For the subacute study, rats will be fasted prior to necropsy. This fasting will not exceed 20 hours.

V.5.1.3. Exceptions:

Animals in the acute study will be singly housed immediately following dosing. If no signs of toxicity are observed during the first 7 ± 2 days, they may be returned to pair housing at the discretion of the PI/SD/study personnel and AV. This is necessary due to the unknown toxicity of the MeNQ. The design of the SSWP results in dosing of one to three animals at each dose with often a wide margin of time between doses. Pair-housing could result in animals co-housed that exhibit very different signs of toxicity or a healthy animal co-housed with animals exhibiting toxicity or morbidity. This could result in undue stress to the healthy animals or loss of data from moribund animals if aggression or cannibalism occurs. Pair-housed animals in both studies may also be separated if there are behavioral changes (e.g., aggression or hyperactivity).

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care:

Animals will routinely be observed no less than once daily by assigned veterinary medical personnel for husbandry conditions, humane care, and general health status[25]. In the event an animal becomes ill or injured, veterinary or toxicology personnel will contact the AV or his/her designated backup who will determine the appropriate course of action. Animals will also be observed daily by study personnel as described in sections V.1.1 and V.1.2. This consists of a twice daily observation on duty days and at least once daily on non-duty days by assigned veterinary medicine personnel and/or study personnel. Observations by study personnel will be taken approximately 4-hours or later following dosing. In consultation with the AV and using a weight of evidence approach, the continued necessity of 4-hour post-dose observations on non-duty days will be assessed. The weight of evidence approach includes factors such as: 1) a general lack of compound toxicity; 2) a lack of other clinical signs of illness; and 3) no distressed animals that are likely to require some form of intervention in less than a 24-hour period. Only the AV can authorize the suspension of the second observation on non-duty days. Animals appearing ill or displaying signs of toxicity will be assessed for moribundity and early removal from the study as described in section V.4.5. For illnesses unrelated to the administration of the test compound, the AV will discuss the plan for care with the PI/SD prior to initiation of any intervention.

V.5.2.2. Emergency Veterinary Medical Care:

In the event an animal requires after-hours emergency veterinary care, a veterinarian is available 24 hours a day, 7 days a week. In the case of an emergency health problem,

if the PI/SD or co-PI is unavailable or the investigator staff and veterinary staff cannot reach consensus on treatment of a study animal, the veterinarian has the authority to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary. However, all decisions involving the treatment of a study animal in which a consensus cannot be reached will only be made after the veterinarian or designated backup veterinarian has actually observed and examined the animal in question. To facilitate communication, the animal care staff will maintain an emergency contact roster. In an emergency, the animal care staff will phone the numbers (office, home, and mobile) listed for the PI/SD and co-PI. If the PI/SD or co-PI cannot be reached by phone within 15 minutes, then they are considered unavailable.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy:

All animals, except during the acute study following dosing, will be pair-housed. Animal enrichment such as a nylabone and a rodent retreat will be provided in their cage. All animals will receive the same type of enrichment. There will be an environmental enrichment plan posted on the door of the animal room to communicate the enrichment plan to everyone working on the protocol. This is in accordance with VMD SOP 004[20]. Additionally, the PI/SD, co-investigators or veterinary staff will handle rats several times per week during the acclimatization period and prior to test article exposure to acclimate them to handling prior to the start of study. Other enrichment items/activities may be added as approved by the AV.

V.5.3.2. Enrichment Restrictions:

Acute study animals will be singly housed following dosing as described in section V.5.1.3. Food enrichment will be restricted due to the need to fast acute study animals prior to dosing and the need to fast animals prior to necropsy in the 14-day study. As such, rodent chow blocks will not be placed on the cage floor for animals. Rodent retreats may be removed for observation of animals, but will be replaced following observation periods of no more than eight hours.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Personnel on Protocol	Activities to be performed on this protocol	Formal Training	Qualifications & Experience
Emily Reinke	Handling/observations	Rat handling (6/12/12)	Ph.D., Pathology; M.S, Animal Sciences 4+ yrs Animal Research Experience
	Oral gavage	Rat oral gavage (6/12/12)	
	CO_2 euthanasia	Rat euthanasia (6/12/12)	
	Blood Collection	Saphenous and tail vein bleeding (5/28/14)	
Lee Crouse	Handling/observations	Rodent handling techniques (11/21/96);	M.S., Environmental

		Rat handling (7/19/07)	Science 16+ Yrs Animal Research Experience
	Oral Gavage	Rat gavage (07/19/07); Rat oral gavage (05/05/08); rat oral gavage (03/03/08); rat oral gavage, 14 day (05/01/09)	
	Blood collection	Rat bleeding techniques: cardiac under isoflurane (12/17/08); rat blood collection (7/19/07); Terminal cardiac blood draw (5/1/09); Survival Bleeding (TBS)	
	CO_2 euthanasia	Rat euthanasia via CO_2 (7/19/07; 5/01/09)	
Michael Quinn	Handling/observations	Rodent small animal handling workshop (6/21/05); Rodent handling techniques (6/30/11)	Ph.D., Animal Science 16+ Yrs Animal Research Experience
	Oral Gavage	Rat oral gavage (6/2012)	
	CO_2 euthanasia	Rodent small animal handling workshop (6/21/05)	
	Blood Collection	Saphenous Bleeding (6/6/13); Survival Bleeding (TBS)	
Valerie Adams	Handling/observations	Rat techniques: handling and observations (11/3/08); Small animal handling workshop (5/28/09)	Ph.D., Cell and Structural Biology 10+ Yrs Animal Research Experience
	Oral Gavage	Rat techniques: handling and observations (11/3/08)	
	Blood collection	Rat techniques: basic bleeding (11/03/08); Small animal handling workshop: Intracardiac bleed (5/28/09); Saphenous Bleeding (6/6/13); Saphenous and Tail Vein Bleeding (5/28/14)	
	CO_2 euthanasia	Small animal handling workshop: euthanasia (5/28/09); Rat training:	

		CO_2 euthanasia (6/24/09)	
Emily Lent	Handling/observations	Rat handling (7/19/07)	Ph.D., Natural Resources and Environmental Studies; M.S., Wildlife Biology 13+ Yrs Animal Research Experience
	Blood collection	Rat bleeding techniques (7/19/07; 4/30/08); Survival Bleeding (TBS)	
	CO_2 euthanasia	Rat euthanasia via CO_2 (7/19/07; 11/18/10)	
Theresa Hanna	Handling/observations	Animal handling: rat (3/12/92); rat techniques: handling/observations (11/3/08); Rodent small animal handling workshop (2/25/98; 4/2/04; 11/22/05)	ALAT 15+ Yrs Animal Research Experience
	Blood collection	Rat techniques: basic bleeding (11/3/08; Rat terminal cardiac blood draw (5/1/09); Saphenous Bleeding (6/6/13); Survival Bleeding (TBS)	
	CO_2 euthanasia	Rat euthanasia CO_2 (3/27/09); Rat CO_2 euthanasia (5/1/09)	
Allison Jackovitz	Handling/observations	Small animal handling workshop (6/4/09); Rat handling (6/12/12)	B.S., Biology 3+ Yrs Animal Research Experience
	Oral Gavage	Rat oral gavage (6/12/12); Rat oral gavage (6/19/12)	
	CO_2 anesthesia/cardiac blood collection	CO_2 anesthesia (5/2/13) cardiac blood collection (5/8/13) CO_2 euthanasia (5/10/13)	
	CO_2 euthanasia	Small animal handling workshop: euthanasia (6/4/09); Rat CO_2 euthanasia (6/12/12)	
	Blood Collection	Saphenous Bleeding (6/6/13); Survival Bleeding (TBS)	
Mark Way	Handling/observations	Rodent and small animal handling workshop (5/17/07); Rat handling (7/19/07; 7/9/09)	B.S., Biology AALAS-LAT 17+ Yrs Animal Research Experience

	Oral Gavage	Rodent and small animal handling workshop (5/17/07);	
	Blood collection	Rat techniques: blood collection (7/19/07); Peripheral Bleeding (7/2009); Survival Bleeding (TBS)	
	CO_2 euthanasia	Rat techniques: euthanasia (7/19/07); CO_2 euthanasia (7/9/09)	
Bill Eck	Handling/observations	Rat handling (7/19/07): Small animal handling workshop (5/28/09)	Ph.D., Biochemistry 8+ Yrs Animal Research Experience
	Oral Gavage	Small animal handling workshop: oral gavage in rats (5/28/09)	
	Blood collection	Rat techniques: blood collection (7/19/07); Small animal handling workshop: IC bleed in rats (5/28/09)	
	CO_2 euthanasia	Rat techniques: euthanasia (7/19/07); Small animal handling workshop: CO_2 euthanasia (5/28/09)	
Alicia Shiflett	Handling/observations	Rat techniques: handling/observations (11/3/08): rat handling (6/12/12)	B.S., Public Health 3+ Yrs Animal Research Experience
	Blood collection	Rat techniques: basic bleeding (11/3/08); Survival Bleeding (TBS)	
	CO_2 euthanasia	Rat CO_2 euthanasia (3/27/09)	
Larry Williams	Handling/observations	Rat techniques: handling and observations (11/3/08); Small animal handling workshop (5/28/09); Rat training: handling/observations (6/24/09)	Ph.D., Anatomy 30+ Yrs Animal Research Experience
	Blood collection	Rat techniques: basic bleeding (11/03/08); Small animal handling workshop: IC bleed (5/28/09)	

	CO2 euthanasia	Small animal handling workshop: euthanasia (5/28/09); Rat training: CO2 euthanasia (6/24/09)	
Matt Bazar	Handling/observations	Rodent handling workshop (2/17/04); Rodent and small animal handling workshop (12/7/04); Small animal handling workshop (8/28/09)	M.S., Biology 8+ Yrs Animal Research Experience
	CO_2 euthanasia	Rat CO_2 euthanasia (11/18/10); Small animal handling workshop: euthanasia tech. (8/28/09)	

VII. BIOHAZARD/SAFETY:

Risks associated with this protocol include bites/scratches/needle sticks, transmission of zoonotic diseases, and the development of animal allergies. To minimize risk, appropriate handling techniques will be used and appropriate personal protective equipment (PPE) will be worn for all animal handling work. This includes (but may not be limited to) facemask, gloves, and disposable lab coat. Personnel will wash their hands upon completion of animal work. Applicable current TOX SOPs and PHC regulations (TOX SOP 046.002[26] and USACHPPM 385-5[27], OHS of Animal Users) will be followed. These documents specify hazardous waste disposal, bite/scratch procedures, and zoonotic disease prevention. A sharps container will be present at all times when using sharps and needles will not be recapped after entering animal tissue.

VIII. ENCLOSURES:

References (Appendix A)

IX. ASSURANCES:

IX.1. As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordination regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely, "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: (*Applicable if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.*)
I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL / WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

I. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Policy Memorandum No. 8 "Policy on Unexpected Adverse Event Reporting".

Emily N. Reinke	Emily N. Reinke	7-2-14
(PRINT) Principal Investigator	(Signature)	(Date)

IX.2. As the Primary Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as the Primary Co-Investigator, I am authorized and responsible for performing all procedures and manipulations as assigned to the SD/PI in the SD/PI's absence. This includes euthanasia of distressed animals.

C. Training: I verify that I am technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL or WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

F. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Policy Memorandum No. 8 "Policy on Unexpected Adverse Event Reporting".

Lee CB Crouse

(PRINT) First name, MI, Last name of Primary Co-Investigator

(Signature) 3 July 2014 (Date)

IX.2. As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as a Co-Investigator, I am authorized, responsible for, and willing to perform all procedures and manipulations as assigned to me by the SD/PI.

C. Training: I verify that I am technically competent and have been or will be properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the assigned procedures/manipulations performed by me.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to participate in this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am participating in biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. I will follow the direction of the SD/PI relative to potential pain and/or distress and relief by use of anesthetics, analgesics, and/or tranquilizers.

F. Unexpected Adverse Events: I acknowledge the responsibility for reporting unexpected adverse events IAW the most current version of IACUC Policy Memorandum No. 8 "Policy on Unexpected Adverse Event Reporting".

Michael J. Quinn, Ph.D.		7/7/14
(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

Valerie H. Adams, Ph.D.		7/3/2014
(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

(PRINT)	(SIGNATURE)	(DATE)
First name, MI, Last name of Co-Investigator		

APPENDIX A

References

1. USAPHC, *Environmental Health Assessment for Work Unit CA 06-01, Formulation of New, Environmentally Benign Explosives*. 2010.
2. USAPHC, *IACUC SOP 1.2 Standing Operating Procedures for Animal Use Protocol Development, Submission, Review and Consideration by the IACUC.* 2014, AIPH, USAPHC: Aberdeen Proving Ground, MD.
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21. USAPHC, *VMD SOP No. 016.000, Test System Observations*. 2013, AIPH, USAPHC Aberdeen Proving Ground, MD.
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Referenced SOPs

SOP Long Title	New SOP Number	Previous SOP Number
Clinical Chemistry Analysis of Blood Specimens	TOX SOP 005.001	TOX SOP 005.000
Cell-Dyn Hematology Analyzer	TOX SOP 013.001	TOX SOP 013.000
Animal Health Technician Duties in Animal Rooms	VMD SOP 008.001	TOX SOP 022.000
Individual Animal Identification	VMD SOP 014.000	TOX SOP 024.000
Animal Bleeding Technique	VMD SOP 015.000	TOX SOP 025.000
Test System Observations	VMD SOP 016.000	TOX SOP 026.000
Animal Euthanasia	VMD SOP 002.000	TOX SOP 027.000
Animal Environmental Enrichment	VMD SOP 004.000	TOX SOP 033.000

PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER: - 30 - 14-07-01 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (*Rattus norvegicus*).		
1. SCIENTIFIC MERIT (PEER REVIEW)			
1a. Printed Name (First, MI, Last) Arthur J. O'Neill	1b. Title Program Manager, TEP	1c. Signature ONEILL.ARTHUR.J.III.1299508445	1d. Date (yyyy/mm/dd) 20140529
2. DIRECTOR			
2a. Printed Name (First, MI, Last)	2b. Title	2c. Signature Click to Approve	2d. Date (yyyy/mm/dd)
3. PROGRAM MANAGER			
3a. Printed Name (First, MI, Last)	3b. Title	3c. Signature Click to Approve	3d. Date (yyyy/mm/dd)
4. ATTENDING VETERINARIAN			
4a. Printed Name (First, MI, Last) Dawn C. Fitzhugh	4b. Title Attending Veterinarian	4c. Signature FITZHUGH.DAWN.CATHERINE.1036926127	4d. Date (yyyy/mm/dd) 20140528
5. ANALYTICAL CHEMISTRY (If Applicable)			
5a. Printed Name (First, MI, Last)	5b. Title	5c. Signature Click to Approve	5d. Date (yyyy/mm/dd)
6. SAFETY MANAGER			
6a. Printed Name (First, MI, Last)	6b. Title	6c. Signature Click to Approve	6d. Date (yyyy/mm/dd)
7. STATISTICIAN (If Applicable)			
7a. Printed Name (First, MI, Last) Karen D. Deaver	7b. Title Senior Command Statistician, USAPHC	7c. Signature DEAVER.KAREN.DEVILBISS.1400519672	7d. Date (yyyy/mm/dd) 20140519

PROTOCOL NUMBER: - 30 - 14-07-01 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (Rattus norvegicus).		
8. SIO-QAT (GLP COMPLIANCE AND QA SUPPORT)			
8a. Printed Name (First, MI, Last)	8b. Title	8c. Signature: Click to Approve	8d. Date (yyyy/mm/dd)
9. CHAIRMAN, IACUC			
9a. Printed Name (First, MI, Last): Kristin T. Newkirk	9b. Title: Animal Care and Use Specialist	9c. Signature: NEWKIRK.KRISTIN.TORELL.1014786895	9d. Date (yyyy/mm/dd): 20140702
10. INSTITUTIONAL OFFICIAL			
10a. Printed Name (First, MI, Last): John J. Resta	10b. Title: Director, AIPH	10c. Signature: RESTA.JOHN.J.1229129305	10d. Date (yyyy/mm/dd): 20140710
11. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR			
11a. Printed Name (First, MI, Last): Emily N. Reinke	11b. Title: Biologist	11c. Signature: REINKE.EMILY.NICOLE.1447560620	11d. Date (yyyy/mm/dd): 20140714
12. OTHER ORGANIZATION(S) PROVIDING SUPPORT (AS NEEDED):			
12a. Printed Name (First, MI, Last)	12b. Title	12c. Signature	12d. Date (yyyy/mm/dd)
13. STUDY SPONSOR:			
13a. Printed Name (First, MI, Last): for MARK S. JOHNSON	13b. Title: DIRECTOR, TOXICOLOGY	13c. Signature	13d. Date (yyyy/mm/dd): 20160510

CHPPM FORM 445-R-E, March 2008

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2014/07/10	2. PROTOCOL NUMBER: 30-14-07-01	3. MODIFICATION#: 1

4. PROTOCOL TITLE: Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Emily N. Reinke, Ph.D.	410-436-2896	MCHB-IP-THE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY:									1b. N/A ☑
2. ORIGINAL PROTOCOL TOTAL: 185					3. PROTOCOL TOTAL AFTER MODIFICATION: 185				
2a. USDA pain cat:	B: 0	C: 30	D: 60	E: 95	3a. USDA pain cat:	B: 0	C: 30	D: 60	E: 95

4. Yes	No	
☐	☑	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☑	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☑	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	**SECTION III. MODIFICATION/JUSTIFICATION** *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
Page 8, Section V.1.2. Experiment 2	1. MODIFICATION: Pg. 8, paragraph 3, sentence 9, change 4 to 5 to read: "The 5 MNA negative/positive control rats are subdivided prior to dosing..."
	1a. JUSTIFICATION/REASON: 4 MNA animals listed for use in the experiment 2 description is incorrect. The correct number is 5, as indicated in the summary of animal use table on page 6. This is an editorial error that was discovered after final approval of the protocol.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
Page 9, Section V.1.2. Experiment 2	2. MODIFICATION: Pg. 9, paragraph 2, remove "lungs" from the list of organs harvested. Sentence 3 should read: "At minimum, the following tissues will be harvested, weighed and preserved in 10% formalin: brain, heart, kidneys, liver, spleen and thymus."
	2a. JUSTIFICATION/REASON: Lungs are not weighed at necropsy.
P17, Section V.4.4.8.2. Micronucleus Assay	3. MODIFICATION: Pg. 17, paragraph 2, sentence 1, change 4 to 5 to read: "During the subacute portion of the MeNQ study, 5 additional male rats..." Pg. 17, paragraph 2, sentence 3, change 4 to 5 to read: "In order to get a negative control sample, the 5 male rats will be left untreated..."
	3a. JUSTIFICATION/REASON: 4 animals listed for use in the micronucleus assay description is incorrect. The correct number is 5, as indicated in the summary of animal use table on page 6. This is an editorial error that was discovered after final approval of the protocol.
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☑

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Emily N. Reinke, Ph.D.	Signature Emily N. Reinke	DATE: (yyyy/mm/dd) 20140710
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) KRISTIN T. NEWKIRK	APPROVED / REVIEWED YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 20140721

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2014/08/06	2. PROTOCOL NUMBER: 30-14-07-01	3. MODIFICATION#: GLP-1

4. PROTOCOL TITLE: Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Emily N. Reinke, Ph.D.	410-436-2896	MCHB-IP-THE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Adjustment to animal numbers for MNA to correlate with approved tables. Correction for tissue types to be collected and weighed during necropsy.		21 Jul 2014

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 1b. N/A ☑

2. ORIGINAL PROTOCOL TOTAL: 185 3. PROTOCOL TOTAL AFTER MODIFICATION: 185

2a. USDA pain cat:	B: 0	C: 30	D: 60	E: 95	3a. USDA pain cat:	B: 0	C: 30	D: 60	E: 95

4. Yes	No	
☐	☑	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☑	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☑	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	**SECTION III. MODIFICATION/JUSTIFICATION** *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
Page 8, Section V.1.2 Experiment 2	1. MODIFICATION: Pg. 8, paragraph 3, sentence 2 change 'Day -3' to 'Day -4' for sentence to read: 'Weights will be taken on day -4 (for general health check), -1 (randomization)....' 1a. JUSTIFICATION/REASON: The first weights for the rats are taken as a general health check. The day on which these weights are taken is not critical so long as it is prior to the start of the study. Adjusting the first weight check to -4 days allows for the weights to be taken on a normal duty day, instead of requiring staff to come in on the weekend to take a weight recording. Page 19, Section V.5.1. Husbandry Considerations has the provision for study personnel to weigh the rats during their acclimatization period, so long as they are not handled or weighed within the first 24-hours of arrival into the animal facility.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
	2. MODIFICATION:
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☑

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Emily N. Reinke, PhD, Study Director	Signature Emily N. Reinke	DATE: (yyyy/mm/dd) 20140807
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Michael P. Kefauver, Quality Systems and Regulatory Compliance Office, Quality Assurance Unit (QAU)	APPROVED (REVIEWED) YES ☑ NO ☐ Signature Michael P. Kefauver	DATE: (yyyy/mm/dd) 20140807

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2015/01/28	2. PROTOCOL NUMBER: 30-14-07-01	3. MODIFICATION#: GLP-2

4. PROTOCOL TITLE: Effects of Acute and Subacute Oral Methylnitroguanidine (MeNQ) Exposure to Rats (Rattus norvegicus)

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Emily N. Reinke, Ph.D.	410-436-2896	MCHB-IP-THE

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX)
1	Adjustment to animal numbers for MNA to correlate with approved tables. Correction for tissue types to be collected and weighed during necropsy.		21 Jul 2014
GLP-1	Adjustment to days on which animals will be weighed prior to the initiation of dosing.		7 Aug 2014

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY:						1b. N/A ☐			
2. ORIGINAL PROTOCOL TOTAL:					3. PROTOCOL TOTAL AFTER MODIFICATION:				
2a. USDA pain cat:	B:	C:	D:	E:	3a. USDA pain cat:	B:	C:	D:	E:

4. Yes	No	
☐	☑	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☑	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☑	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	**SECTION III. MODIFICATION/JUSTIFICATION** *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
Page 10, Section V.2 Sample Size Evaluation, Data Analysis Plan and Archiving of Data	1. MODIFICATION: Pg. 8, paragraph 1, sentence 8. Adjust whole sentence to read: Absolute organ weights will be analyzed by a one-way ANOVA, organ to brain and organ to body weight ratios will also be calculated and analyzed by one-way ANOVA.
	1a. JUSTIFICATION/REASON: The use of the ANOVA for absolute organ weights and for the organ weight ratios is statistically acceptable in lieu of using the ANCOVA with body weight as the covariate.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
Page 10, Section V.2 Sample Size Evaluation, Data Analysis Plan and Archiving of Data	2. MODIFICATION: Pg. 8, paragraph 1, sentence 11: Remove ANCOVA to read: ...prior to ANOVA/ANCOVA, or analyzed...
	2a. JUSTIFICATION/REASON:
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Emily N. Reinke, Ph.D.	Signature Emily N. Reinke	DATE: (yyyy/mm/dd) 20150128
2. PROGRAM MANAGER:: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
3. ATTENDING VETERINARIAN: (Printed Name)	Signature	DATE: (yyyy/mm/dd)
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) Michael P. Kefauver, Quality Systems and Regulatory Compliance Office	APPROVED / REVIEWED YES ✓ NO ☐ Signature Michael P. Kefauver	DATE: (yyyy/mm/dd) 20150128